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VOLUME XVI JULY, 1924 No. 3

FURTHER OBSERVATIONS ON THE EFFECTS OF DIRECT TRANSMISSION ON TRYPANOSOMES.

BY H. LYNDHURST DUKE, O.B.E., M.D., Sc.D. (CANTAB.).

THE following brief communication presents another instalment of experiments on the effects of prolonged direct transmission upon trypanosome strains kept up in the laboratory.

In a paper recently published in this Journal two strains of the polymorphic trypanosome from the northern tsetse-zone of Lake Victoria were studied in relation to direct transmission (Duke, 1923). Known as the "Direct Transmission Strain" and the "Antelope Strain," they were recovered respectively from wild lake-shore *Glossina palpalis* and from a situtunga antelope shot on Damba Island on Lake Victoria. Both these strains were maintained at the Entebbe Laboratory by direct transmission from mammal to mammal for a period of over two years. During that period they both underwent considerable changes.

The Direct Transmission Strain, between January, 1920, and October, 1922, was passaged 42 times. During this period the virulence of the trypanosome in laboratory animals increased greatly; at the same time, the power of cyclical development in *G. palpalis* was completely lost.

The Antelope Strain, between September, 1920, and November, 1922, was passaged 29 times. During this time the virulence was slightly increased, but a most interesting change occurred in its behaviour in *G. palpalis*. The power of cyclical development in the tsetse was seriously impaired, but not completely lost. Out of a total of 524 laboratory-bred flies employed in the attempts to transmit this strain, 12 contained developing flagellates; but in every one of these infected flies the flagellates were confined to the gut. Apparently the trypanosome had lost the power to negotiate the final and all-essential stage of its development in the insect, *i.e.* the invasion of the salivary glands.

In the present paper the behaviour of the Antelope Strain under continued direct transmission is studied up to date. These experiments cover the period from November, 1922, to December, 1923, and bring to a close a chapter in the retrogressive history of the strain, still incomplete in November, 1922. The technique pursued is identical with that described in previous papers (Duke, 1921, 1923). The species of monkey used was the greenish-grey, black-faced *Cercopithecus*, used in all previous work at Entebbe. The tsetse flies were hatched in the laboratory.

Table I shows the maintenance of the Antelope Strain by sub-inoculation with the syringe, from November, 1922, to December, 1923.

Table I.

Experiment No.	No. of passage	Duration of disease in days	Day of disease when monkey sub-inoculated
Monkey *568*	28	*30*	*41*
„ *607*	29	*30*	*30*
G.-pig *621*	30	*56*	*24*
Monkey *634* g.-pigs 624 625	31	*22* 16 30	*28* 46 46
G.-pig *637*	32	*31*	*18*
G.-pigs *681* 679	33	*21* 15	*30* 28
„ *701* 700	34	*21* 16	*14* 14
„ *705* 704	35	*35* 29	*16* 16
„ *727* 726 713	36	*9* 9 22	*24* 24 12
Monkey *729*	37	*18*	*9*
„ *739*	38	*18*	*10*
„ *741*	39	*33*	*17*
„ *743* 745	40	*57* 20	*7* 32
„ *747*	41	*17*	*45*
„ *755*	42	*12*	*12*
„ *758* 761	43	*9* 47	*6* 12
Dog *763* 784 (puppy)	44	*29* 25	*9* 33
„ *783*	45	*23*	*29*
Monkey *797*	46	*29*	*22*
Puppy *807*	47	*33*	*6*
Monkey 816	48	*17*	*24*
„ *819* 822	49	Still alive	*10* 15
Dog *823*	50	„	*8*

In Table I the number of the animal from which the serial sub-inoculation was made is in italics, as are also the corresponding numbers in the other columns. Numbers not in italics indicate duplicate inoculations which were not continued.

It will be seen that the virulence of the strain has increased, but that the increase is less striking and less rapid than in the case of the Direct Transmission Strain described in the previous paper (Duke, 1923).

It is difficult to explain the difference in the behaviour of the two strains. From the 30th to 36th passage the Antelope Strain sojourned in guinea-pigs, whereas the Direct Transmission Strain underwent an uninterrupted series of 35 passages in monkeys. Possibly this alternation of hosts has influenced the virulence of the strain.

That a definite increase has taken place in the virulence of the Antelope Strain during the last year is shown by comparing the average duration of the disease in the animals used for the maintenance of the strain before and after November, 1922. For the first 28 passages, between September, 1920, and November, 1922, 37 monkeys and 7 guinea-pigs were employed. The average duration of the disease in the monkeys was 31·4 days, in the guinea-pigs 48·3 days. From the 28th to the 50th passage, *i.e.* from November, 1922, to December, 1923, the average for 14 monkeys is 25·6 days and for 13 guinea-pigs 23·8 days.

We will now turn to the behaviour of the strain in laboratory-bred *G. palpalis*, as set forth in Table II.

Table II.

Expt. No.	Infecting animal	Day on which dissection of flies began	No. of flies alive on 25th day	Flies dissected			Duration of expt. (days)	Day of disease in infecting monkey when flies put on
				Males	Females	Total		
1	Monkey 761	20	47	21	27	48	59	33–34
2	„	19	42	32	16	48	59	34–35
3	„	17	49	36	14	50	55	56, 38
4	„	19	42	25	18	43	50	38–39
5	„	22	40	26	19	45	53	39, 41
6	„	29	56	20	36	56	51	41–42
7	Dog I	20	50	30	22	52	53	10–11, 13
8	„ II	38	53	30	23	53	53	10–13
9	„ I	20	39	18	22	40	52	14–16, 19
10	„ II	12	57	34	26	60	51	14–15, 17, 19
11	„ I	15	54	29	32	61	50	17, 19, 21
12	„ II	18	10	13	3	16	41	19–20
13	Dogs I, II	16	56	45	15	60	46	22, 21
14	Dog I	18	52	24	31	55	44	24–25
15	Monkey 797	11	49	32	24	56	41	6–7
16	„	9	35	37	19	56	41	7–10
17	„	12	63	39	34	73	40	9–10
18	„	8	35	32	19	51	37	11–12, 14–15
19	„	8	45	30	24	54	36	14–15, 17
			874	553	424	977		

In not a single one of the flies in these experiments were any flagellates found.

Similar experiments carried out with this strain at the same time of year in 1922 showed that 2·2 per cent. of the flies used (of a total of 524) had flagellates established in the gut (Duke, 1923).

Morphology. The strain is still polymorphic in character. A striking change has, however, occurred in its morphology. When first isolated from the antelope on Damba Island in September, 1920, posterior-nuclear forms were numerous in guinea-pigs and dogs inoculated with this strain. Thus in one animal, a guinea-pig, 15·2 per cent. of the short trypanosomes showed this peculiarity, these short forms predominating on the slide. At the present time posterior-nuclear forms are hardly ever seen in guinea-pigs and dogs inoculated with the Antelope Strain. Careful search has been made for these forms, and the following is a good sample of the results obtained:

Puppy No. 807 was infected with Antelope Strain on 26. x. 1923. Stained thin blood-films examined on 5, 6, 7, 8, 9, 13, 14, 15, 19, 20, 22, 23, 24, and 27 xi. 1923: 2670 trypanosomes were inspected (200 each day, save on one occasion when only 70 were seen) and only a single trypanosome was seen with the trophonucleus lying in the posterior half—not quarter—of the body: all the others were of normal appearance. In dogs and guinea-pigs alike, true posterior-nuclear forms seem to have quite disappeared.

CONCLUSIONS.

(1) By November, 1922, the Antelope Strain had been maintained at the Entebbe Laboratory by direct transmission through 29 passages over a period of 27 months. During this period the virulence of the strain in laboratory

animals had increased somewhat, and the power of cyclical development in *Glossina palpalis* had been seriously impaired, though not completely lost.

(2) From November, 1922, to December, 1923, the strain has undergone 21 more passages by direct transmission. The virulence has been considerably enhanced, and the power to develop in the tsetse has been lost.

(3) Whereas, when first isolated from the antelope on Damba Island, posterior-nuclear forms were common in guinea-pigs and dogs inoculated with the Antelope Strain, at the time of writing such forms are of exceedingly rare occurrence; indeed, in their typical form, they seem to have quite disappeared.

(4) The behaviour of the Antelope Strain under prolonged direct transmission is thus essentially the same as that previously shown by the Direct Transmission Strain, under similar conditions of maintenance.

(5) It is at present impossible to say whether the loss by the trypanosome of the power to develop in the tsetse is intimately associated with the disappearance of the phenomenon of posterior and anterior displacement of the nucleus.

It is hoped shortly to commence experiments bearing on this point.

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SARCOPTES OF CATTLE.

By A. E. CAMERON, V.S.

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(With 6 Text-figures.)

A YEARLING bull affected with Sarcoptic Mange was shipped to the Veterinary Research Station, Lethbridge, Alberta, and has been a convenient source of supply of mites for study.

The whole of the under part of the head extending up to and including the ears and the lower part of the neck extending back to the brisket had lost most of the hair and was covered with a crust of varying thickness. The skin however had remained comparatively soft and pliable. This animal presented a well established case of Sarcoptic Mange; scrapings taken from the area affected yielded numerous mites. This calf was also heavily infested with lice (*Trichodectes scalaris*).

Outbreaks of Sarcoptic Mange in cattle have occurred in different parts of Canada and its incidence in the United States is probably as great as Psoroptic Mange although the number of cattle affected is less.

This type of Mange has been found in several premises in the Edmonton district of Alberta, from which the above-mentioned yearling was obtained, and although no history of contact could be established in this case there is no doubt the *Sarcoptes* are the same as those seen from other cases in cattle.

The animal was one of eight calves born between March and June, 1922. These were pail fed and confined to a hog lot until about a month before the disease was detected. The calf had never been off the premises and was the only one which showed mange, although the other calves harboured lice. The remaining thirty head of cattle on the farm appeared clean.

Veterinary-Inspector Duthie, who dealt with the case, was of the opinion the disease had been contracted from a Yorkshire sow which had some evidence of skin disease and had shared the same enclosure with other swine and the calves. Inspector Duthie made an examination of scrapings taken from the sow but found no acari. More scrapings were taken and forwarded to me and again the examination was negative. The scrapings were examined dry for living mites and then treated with 40 per cent. sodium hydrate solution and centrifuged. All the scrapings were gone over.

In order that no doubt should exist concerning the identity of the *Sarcoptes* authority was subsequently obtained to purchase another animal from an outbreak in a different locality. It was found, however, that no outbreak of Sarcoptic Mange was being dealt with by the Department of Agriculture and so scrapings taken from the last cases which had been handled were secured from Dr Barker, of Regina, Sask. These were from three widely separated premises although the outbreaks were related in that infection had been traced from one herd to the other. In one outbreak it is reported that 80 head out of a total of 120 cattle were affected; in another four out of 43 cattle were certainly affected while in the last the mites were found on a pure-bred bull, the only animal showing symptoms of mange. In the first lot mentioned in which a large number were involved the cattle were stabled a considerable distance from the hog pens and there were separate attendants. The *Sarcoptes* from these outbreaks have been studied and found to have the same external anatomy as those described. The details are appended.

The specimens dealt with in this paper were taken from the young bull mentioned and are referred to as *Sarcoptes scabiei* var. *bovis*. They were studied and compared with Buxton's data on *Sarcoptes* of the horse and the drawings made have been lettered and numbered in the same way for convenience.

Warburton (1920) has shown that we lacked an accurate knowledge of any of the *Sarcoptes* and since then Buxton (1921) has given a standard to work from in his paper on the external anatomy of the Sarcoptes of the horse.

Comparison of the measurements of *Sarcoptes* by different workers is difficult as the records seldom state whether the mites were alive or mounted when the measurements were made. A record of the same individuals alive and mounted may be of assistance and with this end in view a series of more than sixty specimens from the egg to the adult have been measured in this way. A table showing the measurements is appended. The difference in the measurements is striking. The mounted specimen of a large female has been found to exceed that of the same mite alive by a hundred microns in length and as much in width. The length was taken from the tip of the chelicera to the end of the body and the breadth at the widest part.

The Adult Female.

The average measurement of sixteen specimens alive is 359 microns in length and 261 microns in breadth. Figs. 1 and 2 show the general appearance of the dorsal and ventral surfaces.

The colour of the mite is a dirty white and translucent. The surface glistens if the sun strikes it. In the living state the adult female suggests the appearance of a tortoise. The head and first and second legs are partly covered by a fold of dorsal integument so that the legs appear to be extended from underneath. The portion of the back which carries most of the scales rises a short distance back from the capitulum and inwards from the sides forming a dome which covers the posterior portion of the mite. Along the side of the dome is a

longitudinal furrow on each side. There are also three furrows which run from the base of the dome outwards. Behind the second leg the integument turns under itself slightly forward and forms a deep V-shaped depression. A less pronounced depression occurs behind where the third leg is seen when the mite is moving. In the latter hollow five scales can usually be seen, three of them clearly in profile.

The dorsal surface is largely covered with transverse ridges which follow the different levels marking it like a contour map. On the sides of the posterior

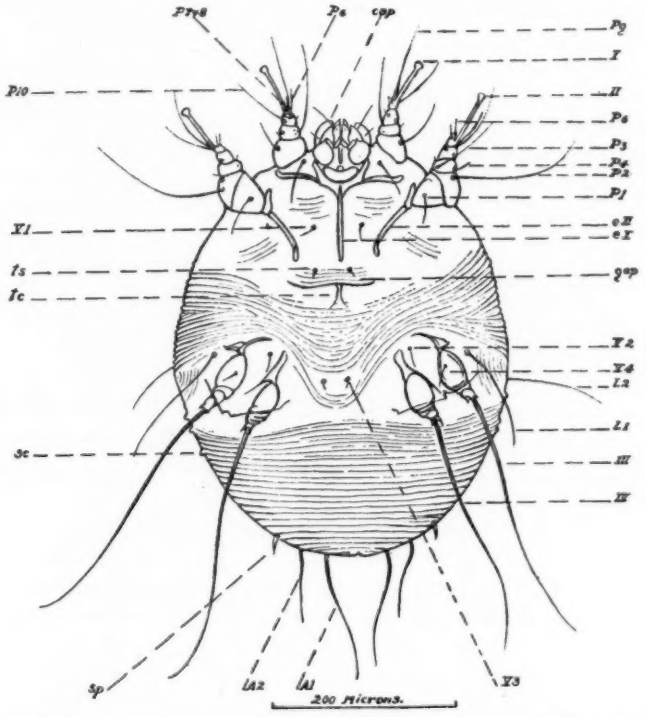

Fig. 1. *Sarcoptes scabiei* var. *bovis*. Adult ♀, ventral surface. *A* 1, *A* 2, anal setae; *cap*, capitulum; *e I*, *e II*, epimeres of legs; *gop*, genital operculum; *L* 1, *L* 2, lateral setae; *P* 1–*P* 10, setae of the legs; *tc*, tocostome; *ts*, tocostomal setae; *V* 1–*V* 4, ventral setae; *I*–*IV*, legs.

portion of the body the ridges are longitudinal. The fold of membrane over the base of the capitulum, the epistome, is free from ridges and has a pitted area from which arise two short setae (*D* 1). The folds of membrane covering the bases of the first and second legs have ridges, and that over the first leg is made more prominent by the dorsal epimere which forms the epaulette (*el*). A short distance posterior is a large oblong transverse area which appears to be depressed and has no ridges. The surface is covered with fine markings;

this area is the plastron (*pl*). Two circular markings just anterior to the plastron are the rudimentary stigmata of Megnin (*st*, Fig. 2). At the posterior border of the plastron, on each side, arise two long setae (*D* 2) which in the living mite are usually turned forwards. On each side just anterior to the middle of the body are situated three cones, acorn-shaped, arising from a base of chitin pointed anteriorly. The scales (*sc*) covering a large part of the surface do not lie flat but are erect and usually point backwards. The tips of the scales are dark coloured. Their shape is difficult to determine but appears

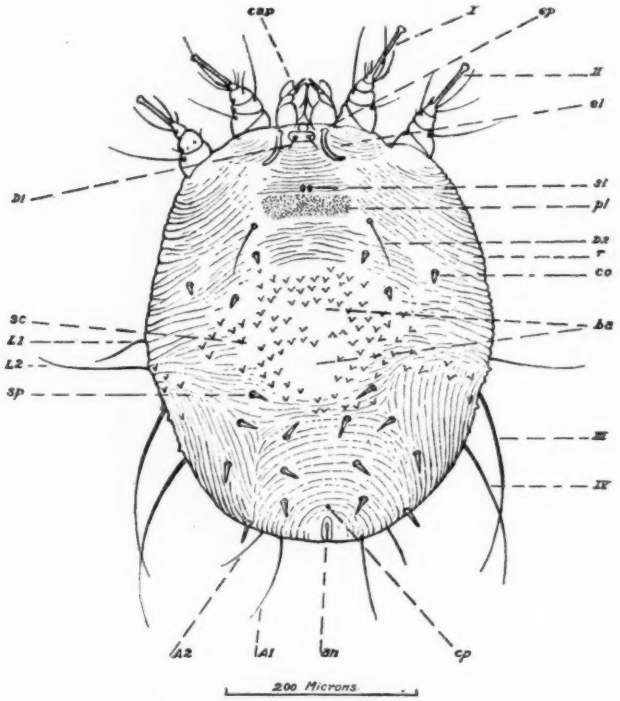

Fig. 2. *Sarcoptes scabiei* var. *bovis*. Adult ♀, dorsal surface. *an*, anus; *A* 1, *A* 2, anal setae; *ba*, bare area; *cap*, capitulum; *co*, cone; *cp*, copulatory papilla; *D* 1, first dorsal setae; *D* 2, second dorsal setae; *el*, epaulette; *ep*, epistome; *L* 1, *L* 2, lateral setae; *pl*, plastron; *r*, ridges of integument; *sc*, scales; *sp*, spine; *st*, rudimentary stigmata; *I–IV*, legs.

like a flattened cone. One hundred and five have been counted on a female but difficulty in counting the scales renders accuracy uncertain. There is a patch near the centre of the dorsal surface free from scales, or with only rudimentary scales, which differs in individuals. Some have this bare area (*ba*) roughly circular while others have the area divided by one or two rows of scales. On the posterior end of the body are seven spines (*sp*) on each side, arranged in two rows, an inner row of three nearly straight and an outer row

of four curved outwards. In *Sarcoptes* of cattle these spines are notched as shown in Fig. 6. When mounted the V-shaped notches may not be seen on all the spines, probably from the position in which they lie. The spines appear to be hollow for at least a part of their length and sometimes contain a reddish-brown substance which may be seen broken into two lots.

The anus is placed in a vertical position at the end of the body, the greater part of it on the dorsal surface. It is a straight longitudinal slit bordered by two lips. In front of the anus is the copulatory papilla. This papilla is small but varies in size in different females. On each side of the anus arise two setae (*A* 1 and *A* 2). From the edge of the body in line with the bare area a long seta (*L* 2) arises.

The ventral surface has transverse parallel ridges covering a large portion of its surface. These become longitudinal at the sides of the third and fourth legs. In the adult female the ridges running backwards between the fourth legs turn and pass forward to the opposite side about the middle of the body, a point of difference from the other stages. The most prominent features on the ventral surface are the epimeres (*e I–IV*). The epimeres of the first legs join to form the straight structure, the sternum, which passes back to just in front of the genital operculum (*gop*). The epimere of the second leg in passing backward is curved and has a small point at the inner side near the end. The epimeres of the third and fourth legs incline towards each other and are much smaller than those of the other legs.

The genital operculum is a fold of integument forming a flap turned forwards and situated transversely just behind the anterior epimeres. Underneath this flap is the tocostome usually seen, just posterior, by transparency. This organ is placed longitudinally and has two lips from which a fine tube passes backward and is just traceable. At some stages of focussing the tocostome (*tc*) resembles a seta.

The setae of the ventral surface are shown in Fig. 1 (*V* 1–4 and *L* 1). The setae *V* 3 between the bases of the fourth legs are situated on the third ridge which turns and passes forward on each side. They have been observed one on the third and one on the fourth ridges so that they were not quite horizontal. Three or four scales are apparent at the sides of the body in line with the fourth legs.

The capitulum has not been dissected out but appears to conform to the description of that of *Sarcoptes* of the horse. There are two setae on the ventral surface and two on the dorsal surface.

The legs. The first and second legs have five joints. There is a noticeable indentation on the inner side of the legs between the first and second joints. The spur, or hook, first described by Megnin, is present on the outer side and under the second joint. This is very noticeable in the living mite examined in water. The terminal joint is provided with two claws and the ambulacrum. This last structure is from two-thirds to three-quarters of the length of the leg proper. The distal extremity of the stem is constricted and has a bell-shaped

sucker attached by its narrow base. The stem of the ambulacrum bends considerably when weight is placed on it by the mite.

The setae of the legs arise from thickenings of chitin and are shown in Fig. 1 (*P* 1–10). They are the same as those described for *Sarcoptes* of the horse except that the two short setae, which do not taper to a point (*P* 7 and 8) on the first leg are represented by a single seta on the second leg of the adult female, while the seta *P* 2 which arises from the outer margin of the second joint on the first leg is not more than half the length of that on the second leg.

The third and fourth legs are concealed from view unless the mite is in motion, when the third leg may be seen at the side of the body if the leg is in use. There appear to be four joints on the third and fourth legs. A fold of integument covers their bases closely and on that covering the third leg is situated the seta *V* 4 which is absent from the same location under the fourth leg. From the terminal joints spring very long setae which can be seen far beyond the margin of the body. On the fourth, terminal, joint of each leg are two claws, a small papilla and four short setae. The four short setae on the end joint of the fourth leg are not quite so long.

The Male.

The average length of eight males measured alive is 234 microns and the breadth 165 microns.

The dorsal surface differs from that of the female in the following details. The plastron in proportion is wider and much longer with the markings more prominent on the posterior half. There are only a few scales on each side. The number varies—from nine to twelve have been counted. Two or three of the scales are on the side far back, about level with the second spine of the lateral row. There are only twelve spines, arranged in rows of three; these are notched at the end as in the female. The cones are more prominent and larger. There are few ridges as compared with the female. There are a pair of posterior plastrons between the inner and lateral rows of spines.

The ventral surface (Fig. 3) shows marked differences from that of the female. The common stem of the first epimeres extends further back. The epimeres of the second legs have two small projections on the inner side where they curve outwards. The ventral setae *V* 1–4, are located as in the female. The setae *V* 3 are larger and more conspicuous. The lateral setae *L* 1 and 2 are present.

The epimeres of the third and fourth legs are united; that of the third leg curves inwards to that of the fourth which is slightly turned outwards. The point of union is reinforced with dense chitin. Between the fourth pair of legs is situated the complex structure forming the genital apparatus. This is supported by a strong chitinous framework, prominent like the epimeres. The central rod running forward ends in a T-shape the arms of which are loosely connected with the united epimeres of the third and fourth legs. The lower part of the genital apparatus has a transparent covering on which are situated

two setae, *V* 5. In Fig. 3 the structures appearing through this genital apron (*ga*) are shown. The genital apparatus of the male Sarcopt of the horse has been figured and described in detail by Buxton and no distinguishing feature has been noted in the *Sarcoptes* of cattle.

The setae of the capitulum are longer in proportion to those of the female.

The legs. The first and second legs are the same as in the female except that the chitinous framework of the legs is denser and the seta *P* 4, from the lateral side of the third joint is longer in the male. The two short setae on the terminal joint of the first leg are also represented by a single seta on the second leg.

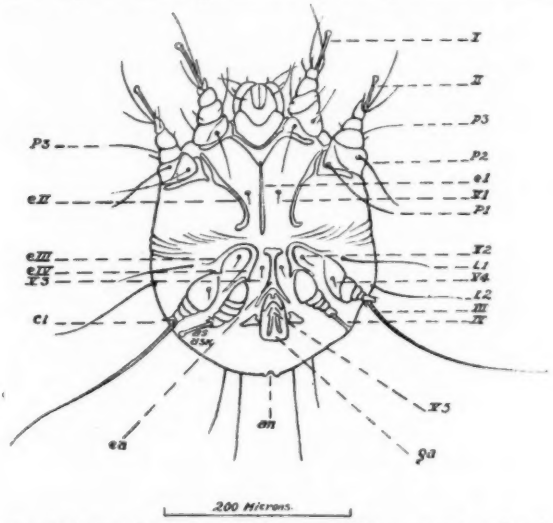

Fig. 3. *Sarcoptes scabiei* var. *bovis* ♂, ventral surface. The structures under the genital apron are shown. The setae *P* 3 on the second legs have passed over the dorsal surface and appear on the opposite side of the legs. *an*, anus; *as*, *ask*, ambulacral stem and sucker; *cl*, claw *ea*, epiandrium; *eI–eIV*, epimeres; *ga*, genital apron; *I–IV*, legs.

The third leg of the male is the same as that of the female but the terminal seta is longer and the short setae are more prominent and thus more easily seen than those of the female. There are two claws, one slightly larger than the other, a small papilla and four short setae on the terminal joint. The fourth leg differs from that in all other stages in having an ambulacrum in place of a terminal seta. This ambulacrum is about half the length of that on the first leg. On the terminal joint there are three short setae, one claw and a large papilla replacing the other claw.

The Immature Female.

Twelve immature females measured alive averaged 234 microns in length and 176 microns in breadth.

The dorsal surface resembles that of the adult with the following differences. The dorsal epimere is not so distinct. The plastron is a bare unmarked area.

The bare area (*ba*) is crossed by distinct ridges (these ridges are faintly marked in the adult). The copulatory papilla has not been seen in any specimen examined.

On the ventral surface are several differences from that of the adult. The posterior ends of the sternum and the epimeres of the second legs are forked. The ridges on which the third ventral setae (*V* 3) are located pass straight back in the immature female and do not turn and pass forward and outward to the opposite side as in the adult.

There is one distinct difference which has not been mentioned in *Sarcoptes* of the horse. At the level of the ends of the fourth legs are two small setae analagous to those on the genital apron of the male (*V* 5).

In other respects the immature female resembles the adult. There are two pairs of lateral setae (*L* 1 and 2) and two pairs of anal setae. The two short setae *P* 7 and 8 on the terminal joint of the first leg are represented by a single seta on the second leg. The posterior legs are the same as those of the adult with four short setae on the terminal joints.

The Nymph.

Nine nymphs measured alive averaged 173 microns in length and 141 microns in breadth.

The dorsal surface shows no scales posterior to the bare area (*ba*) and so differs from the immature female. There are two pairs of anal setae and two pairs of lateral setae (*L* 1 and 2), the latter differing from the description of the nymph of *Sarcoptes* of the horse given by Buxton.

The ventral surface differs from that of the immature female in having no ventral setae *V* 3 and *V* 4 and the setae analagous to *V* 5 in the male.

The first and second legs differ from those of the immature female in having no long setae from the ventral surface of their first joints (*P* 1). The short setae on the terminal joints (*P* 7 and 8) are represented by a single seta on both first and second legs. The fourth leg has no short setae on the terminal joint.

The Larvae.

Eight larvae measured alive averaged 163 microns in length and 117 microns in breadth. The larva has only six legs. The area from which the first dorsal setae (*D* 1) arise does not appear to be sculptured as in the adult but the setae are slightly more prominent. There are three cones on each side as in the other stages (Figs. 4 and 5). Only five spines are present on each side, two in the inner row and three in the lateral row. Two pairs of lateral setae are present, as in all other stages. There is a single seta on each side of the anus. In other respects the larva resembles the nymph.

The Egg.

The average length of ten eggs measured loose on a slide, without compression, is 149 microns and the breadth 95 microns. The development of the larva within the egg is clearly seen when the egg is mounted. One specimen

mounted as an egg (Fig. 4) showed that the larva had emerged between the two cover glasses. The egg was intact after mounting and the larva was only

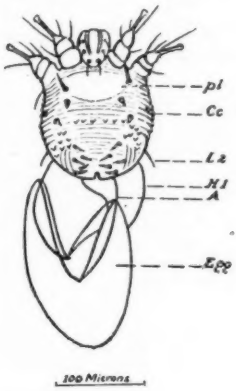

Fig 4

Fig. 6

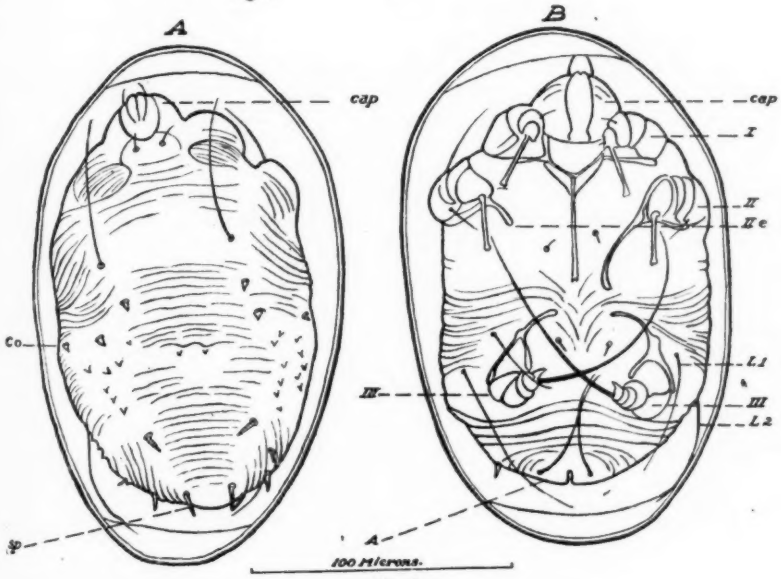

Fig. 5

Fig. 4. *Sarcoptes scabiei* var. *bovis*. Larva just emerged from the egg. *A*, anal seta; *co*, cone; *H* 1, terminal seta of the third leg; *pl*, plastron; *L* 2, lateral seta.

Fig. 5 A and B. *Sarcoptes scabiei* var. *bovis*. Egg containing larva. A. Dorsal surface showing a third, rudimentary, cone. *co*, cone; *sp*, spine. *B*. Ventral surface showing two lateral setae. The epimere of the second right leg is malformed and most of it missing. *A*, anal seta; *cap*. capitulum; *II e*, location where the second epimere should be; *L* 1, *L* 2, lateral setae; *I–III*, legs.

Fig. 6. *Sarcoptes scabiei* var. *bovis*. Spine showing V-shaped termination. (Oil immersion. Not drawn to scale.)

seen to have emerged the following day. This larva shows two lateral setae when viewed on the ventral surface.

Fig. 5 *B* shows the ventral surface of a larva within the egg and the lateral setae *L* 1 and 2 are present. In some eggs in which the larva is well developed there appears to be an air (?) space at the end.

Specimens from other outbreaks of Sarcoptic Mange in Cattle.

A large number of mites from outbreaks in Saskatchewan were examined, but many of these were so dried up and broken that they were of little use for minute examination. A sufficient number were obtained to allow of comparison and with the exception of the nymph fair specimens of all stages were examined.

Eleven adult females mounted measured 375 microns in length and 294 in breadth. Three males were 265 microns in length and 202 microns in breadth. One immature female was 280 microns by 208 microns. Three larvae measured 141 by 100 microns and three eggs 165 by 86 microns.

Some of these are rather less than the measurements of live mites mounted but this is accounted for by the mites from Saskatchewan being dried up when mounted.

Larvae seen in the egg showed three cones and two lateral setae which were also present in all the other stages. The setae *V* 5 were seen in the immature female. There were two short setae on the terminal joint of the first pair of legs, which are represented by a single seta on the second pair, of mature and immature females and the male. The third legs have four short setae on the terminal joint also. No specimen was found in which the setae on the end joint of the fourth pair of legs could be definitely ascertained although two setae were found on several. These setae on the two hind pair of legs are very difficult to see except in the living mite. They are more easily seen if the mite is on its back in water; even this requires much patience.

Technique. Scrapings were taken in petri dishes and if the mites were wanted quickly the scrapings were transferred to small metal boxes with glass fronts. These boxes were carried in the waistcoat pocket as recommended by Goodall. Usually within an hour the mites may be seen on the inner surface of the glass and are easily picked off. If not required until the following day the scrapings were left in the petri dishes. The mites get away from the scab in a few hours.

The small metal boxes mentioned were made from the metal screw tops of cardboard containers—such as are used for mailing bottles or tubes. The portion fixed to the cardboard has a flange which admits of cementing a circular piece of glass inside. The cap of the container is screwed on from the opposite side. The interior, excepting the glass, was blackened. Such boxes are useful in studying the biology of mange parasites.

The medium used for examination of the mites was water and also a gum medium composed of gum arabic 5, saccharose 1, boric acid 1, water 100.

Specimens were mounted between two cover glasses. This admits of viewing both the dorsal and ventral surfaces of the same mite, which has advantages. The cover glasses are placed on a slide for examination. They may be fixed on a slide with balsam with the desired surface up.

The gum arabic medium recommended by Buxton was used for permanent mounts.

The figures, with the exception of Fig. 6, were drawn with a Zeiss camera lucida; some details were added with the aid of the high power.

SUMMARY.

The differences noted between these *Sarcoptes* from cattle and those from the horse, described by Buxton, are as follows:

There are two lateral setae in all stages. The spines are notched or V-shaped at the ends. There are three cones in the larva as well as in all other stages. There is a pair of setae analogous to that found on the genital apron of the male at the corresponding location in the immature female. In the immature female the ridges on which are located the third ventral setae (*V* 3) run backward without returning forward on the opposite side. In the adult female, immature female and male, the two short setae on the terminal joint of the first leg, *P* 7 and 8, are represented by a single seta on the second leg. The third leg in all stages is furnished with four short setae on the terminal joint. The end joint of the fourth leg of both adult and immature females has four short setae. In the nymph none are present on the fourth leg.

Measurements of Sarcoptes scabiei *var.* bovis. (Expressed in microns.)

		Number measured	Range		Average		Ratio Length to breadth
			Length	Breadth	Length	Breadth	
Adult females	Alive	16	305–402	237–285	359	261	100 : 73
	Mounted	16	370–519	281–417	460	349	100 : 76
Males	Alive	8	209–250	149–175	234	165	100 : 71
	Mounted	13	204–301	155–232	250	191	100 : 76
Immature females	Alive	12	187–280	140–197	234	176	100 : 75
	Mounted	9	221–312	175–234	255	200	100 : 78
Nymphs	Alive	9	125–200	91–169	173	141	100 : 82
	Mounted	9	162–276	131–206	204	171	100 : 84
Larvae	Alive	8	137–206	100–135	163	117	100 : 72
	Mounted	8	132–209	107–151	165	128	100 : 78
Eggs	Without compression	10	132–167	87–106	149	95	100 : 64
	Mounted	8	181–223	62–135	199	108	100 : 54

The mounted specimens are, in every case, the same as those measured alive. Five extra males, mounted, are included. Accidents in handling the mites account for the number of mounted specimens being less, in some cases, than that of the living ones measured.

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ON THE COLLECTION AND EXAMINATION OF TAPEWORMS.

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With the exception of the Handbook of Braun and Lühe (1910) and two papers by Gough (1911) and Baylis (1922), very little has appeared dealing with the technique of the examination of cestodes, in particular no special methods for their rapid examination have been described. Fixing, staining and mounting, with the washing necessary at various stages in the preparation of cestodes, may occupy from two to five days. Some other quicker process is obviously needed, this it is the main object of the present paper to supply. The following methods should be understood to apply to fresh material.

Collection.

The collection of the larger cestodes presents no difficulty and may be ignored. To obtain smaller forms, experience has shown the following to be the best procedure: Open the intestine in lengths of approximately 4 inches, one portion at a time, cut off the part opened and shake vigorously in a flat dish (a convenient size is $10 \times 6 \times 2{\cdot}5$ inches) filled with tepid water of approximately 40° C. temperature: if the intestinal contents be not fresh and consequently contain much mucus a slightly higher temperature should be employed. Salt solution or cold water should never be used. Worms which have floated away from the intestine may easily be seen in the water, often moving vigorously. On no account should they be separated by force as rupture of the strobilus with consequent loss of the scolex usually results. After washing in this manner, the intestine with the attached worms should be removed to another similar dish and examined with a powerful lens, preferably a binocular, the scoleces and smaller worms being dissected out with needles. This process should be repeated with each portion of the intestine and, with care, will ensure the collection of 90 per cent. of the cestodes. Scraping the intestine with the back of a scalpel into a gas-jar with subsequent washing the writer has found to be both tedious and ineffective, entailing much additional work and usually causing the fragmentation of each strobilus: the scoleces in particular are often detached and, in the case of the smaller forms, overlooked. Examination under a magnification of 50, drop by drop, of the residue obtained after washing, has never increased the number collected to any appreciable extent. By the first process at least five times as many may

be obtained in a shorter time and a more perfect condition. Unless very accurate cytological work be intended it is always best for the worms to be left in the water for a short time to allow of the relaxation of the musculature.

RAPID EXAMINATION.

For the rapid examination of the scolex, lacto-phenol (carbolic acid, 20 per cent.; lactic acid, 20 per cent.; glycerine, 40 per cent.; water, 20 per cent.) should be used, the scolex being placed alive without any previous treatment, direct from the water into the mounting medium and the coverslip ringed with a mixture of equal parts of Canada balsam and hard wax, melted and applied with a glass rod. Care should be taken that the lacto-phenol does not ooze from the coverslip before ringing or leakage will occur subsequently. Mounted in this way the hooks are clearly defined and can be drawn and identified with great care. The chief disadvantage is that the head, not having been hardened, is usually compressed by the pressure of the cover-glass. To correct this, other scoleces should be killed by hot absolute alcohol and cleared in clove oil, either with or without previous staining: the proportions are correct but the hooks not so clear. Lacto-phenol is also a good mounting medium for gravid proglottides and eggs.

For the examination of the genital organs staining is necessary, the best stain being 97 parts of a saturated solution of carmine in 45 per cent. acetic acid and 3 parts of a saturated solution of ferric acetate in glacial acetic acid, prepared immediately before use. The tapeworm is removed from the dish of water and placed alive in the stain for 5–30 minutes according to its size, 10 minutes giving the best results for *Raillietina echinobothrida* and 5 minutes for *Amoebotaenia sphenoides* and *Hymenolepis carioca*. It can then either be mounted in lacto-phenol or, preferably, be placed for 5 minutes in absolute alcohol and then cleared in clove oil. The result is equal to, or better than that obtained by any other stain but, according to Lee (1921, p. 138) the preparations are not permanent.

PRESERVATION.

All fixatives, with the exception of alcohol, should be used cold. Zenker's fluid (corrosive sublimate, 5 grm.; glacial acetic acid, 5 c.c.; potassium bichromate, 2 grm.; distilled water, 100 c.c.) is undoubtedly the best but must not be allowed to act for more than 24 hours and subsequent prolonged washing (24 hours) in running water is necessary. Contrary to the usual opinion, the writer has had no ill results from the employment of metallic instruments for the handling of objects, care being taken not to leave the instrument long in the fluid and to wipe it at frequent intervals. Bouin's fluid (saturated aqueous solution of picric acid, 75 parts; formol, 25 parts; acetic acid, 5 parts) is not as good as the previous fixative, requires more washing, but material may remain immersed for longer periods.

The cestodes should be fixed in the manner described by Baylis (1922, p. 407): "...by picking up each worm by the end remote from the scolex and

allowing it to hang down, when its own weight will usually cause it to stretch sufficiently. It may then be dipped quickly several times into a jar of the fixing fluid. Small forms, whose weight is not sufficient to stretch them, may be drawn along the edge of the vessel after each dipping so as to exert a slight longitudinal pull on the strobilus." Minute forms (*e.g. Amoebotaenia sphenoides*) should be pipetted into the fixative. The writer has had no good results by other methods. When immediate examination of the intestinal contents is not possible, the intestine should be slit open and either placed directly or have the contents scraped into a jar of water containing a few lumps of corrosive sublimate. Cestodes will remain in a sufficiently good condition for identification for a year, although naturally the fixation is not equal to that obtained by the preceding methods.

Permanent Preparations.

The stains employed differ according to the worker and here no attempt will be made to discuss them. The writer has obtained the best results from haematoxylin, either Delafield's or Ehrlich's acid haematoxylin, a few drops tingeing a tube of distilled water to a faint purple colour. After 24 hours the specimen is placed directly into acid alcohol and differentiated until, in all but minute specimens, the genitalia can be seen by transmitted light. Equally good results have been obtained by acetic alum carmine (excess of carmine in saturated solution of potassium alum, plus 10 per cent. glacial acetic acid), the concentrated solution being diluted ten times and allowed to act for 24 hours. With sections, counterstaining may be obtained by the use of haematoxylin for the genital organs and an acid solution of orange *G* for differentiation and general plasma stain.

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STUDIES ON THE ANATOMY OF *CIMEX LECTULARIUS* L. II.

THE STINK ORGANS.

By I. M. PURI, M.Sc. (Punjab, India).

From the Molteno Institute for Research in Parasitology.

(With 6 Text-figures.)

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Introduction.

A large majority of Hemiptera-Heteroptera are characterised by a very offensive smell. It is due to a malodorous fluid secreted by the dorsal abdominal glands in the larvae and the nymph and by a ventral thoracic glandular organ in the adult.

In *Cimex lectularius* L., Dufour (1833) first described the odorific organ of the adult as a median, oval or rounded, membranous bag lying in the thoracic region of the insect. This bag, according to Dufour, opens outwardly by means of small apertures lying one on each side of the metasternum between the second and the third pairs of legs. He considered this bag as the reservoir and admitted that he could not discover the real gland which secretes the fluid.

Landois (1868) described this organ as composed of a hollow kidney-shaped gland opening into a small median chamber formed by the union of two elongated sacs. The central chamber, according to him, opens outwardly through a very fine median ventral aperture.

Künckel d'Herculais (1886) found that there is a great difference in structure and position of odorific organs between the larva and the adult. In the latter, he says, they are formed of a pair of elongated sacs communicating with a median chamber which opens to the outside by means of a pair

of apertures lying on the metasterunm. Instead of Landois' (1868) kidney-shaped gland, he describes two groups of glandular caeca, placed laterally within the wall of the median chamber.

Patton and Cragg (1913) have not made personal observations on the stink-apparatus of *Cimex rotundatus* but only quote Landois' results.

According to Murray (1914) the stink-apparatus is composed of: (1) two sac-like glands (the reservoirs of previous writers) opening to the exterior separately on the metasternum, and (2) a median chamber opening independently by means of a very fine median metasternal pore. The median kidney-shaped structure, lying in the central chamber, he considers to be an olfactory sense organ and not a gland as described by Landois.

This brief survey of the literature shows how very different are the views held by writers as to the identity of the glandular portion of this organ. My personal observations lead me to the conclusion that all these writers have missed the real gland and have been trying to assign secretory functions to various other non-glandular portions of the stink-apparatus.

Structure of Stink-apparatus of the Adult Bug.

On opening the body-cavity of an adult bed-bug and removing the alimentary canal, the stink-apparatus (Fig. 1) is seen in the region of the metathorax, lying slightly posterior and ventral to the thoracic nerve ganglion. It is composed of a pair of branching glands (*G*), a central reservoir (*C.R.*) with two posteriorly-directed sac-like diverticula—the lateral reservoirs (*L.R.*), a pair of external openings (*O*), and a median kidney-shaped organ (*K*).

The Glands[1]. The stink-glands proper, which have been overlooked by the writers quoted, are a pair of branched structures lying one on each side of the body, more or less covering the reservoirs dorsally. They can easily be distinguished from the fat-body by their paler colour.

From each gland there arises a short thick *main-duct* (*m.d.*) which opens into the lateral region of the central reservoir. This duct gives rise to a number of branches radiating in one plane and dividing ultimately into secondary branches, which form *collecting-ducts* (*c.d.*, Fig. 2) of the terminal lobes of this gland. The duct has a uniform structure throughout its length, from its origin to its ultimate ramification in the gland. The wall of the duct is composed of a layer of thick transversely wrinkled chitin, lined on the outer side by a layer of very small epithelial cells (*e*, Fig. 3). The large gland-cells (Figs. 2 and 4) surround the branches of this duct in a single compact layer. Each cell is a complete unicellular gland with an *intraprotoplasmic ductule* (*i.d.*), opening into the ramified collecting-duct. The intraprotoplasmic ductule is a very fine chitinous tube, the end of which is closed and slightly dilated to form a secretory vesicle. Around this closed end the protoplasm of the cell shows a

[1] From the position that these glands occupy in the body of the bug, it seems very probable that Landois (1868) may have wrongly described a part of them as his third type or "*tubular*" *salivary glands* (see Puri, I. 1924, p. 89).

clear space (*c.z.*) surrounded by another layer of protoplasm, which stains a deeper colour than the rest of the cell. Beyond this uniformly staining area the granular protoplasm begins. Each of these cells has a single large nucleus (*N*) with a well-marked nucleolus.

It appears therefore that each stink-gland is formed by the aggregation of unicellular glandules and must consequently be considered as a multicellular

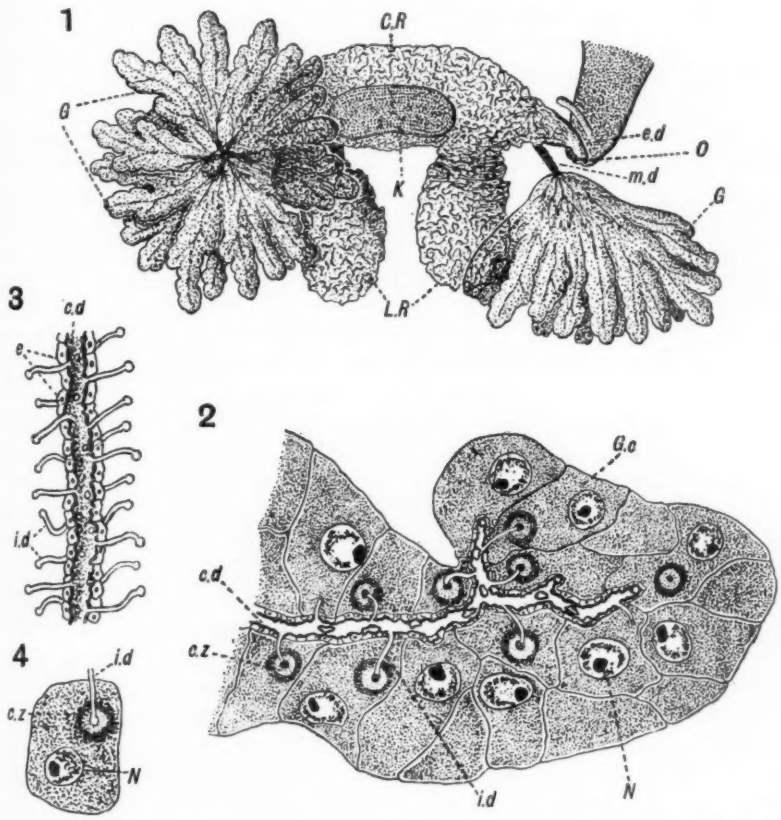

Fig. 1. Stink-apparatus of adult *Cimex lectularius*, from dorsal side, with the gland of the right side displaced. (Slightly reconstructed.) ×86.

Fig. 2. A part of longitudinal section of stink-gland. (Stained with Mann's solution.) ×1240.

Fig. 3. An optical section of a part of *collecting duct* and also showing intraprotoplasmic ductules. (Diagrammatic.)

Fig. 4. A single gland-cell from the stink-gland. (Diagrammatic.)

c.d. Collecting duct. *C.R.* Central reservoir. *c.z.* Clear area surrounding the secretory vesicle. *e.* Epithelial cells lining the collecting duct. *e.d.* Efferent duct. *G.* Stink-gland. *G.c.* A gland cell. *i.d.* Intraprotoplasmic ductules. *K.* Kidney-shaped organ. *L.R.* Lateral reservoirs. *m.d.* Main duct. *N.* Nucleus. *O.* External opening of the stink-apparatus.

compound gland, belonging to the same type as the "*Pluricellulari Composte*" of Berlese (1909).

The Reservoirs. The *central reservoir* (*C.R.*) is composed of an elongated sac stretching from side to side of the insect and is provided with a kidney-shaped thickened area (*K*) in its posterior wall.

Laterally the central reservoir gives off two backwardly-directed diverticula. These are the *lateral reservoirs* (*L.R.*), which Murray (1914), unlike all the previous writers on the subject, erroneously calls "the glands." The shape and size of these reservoirs, especially of the lateral ones, depends on the extent to which they are filled with the secretion. Sometimes the lateral diverticula are so distended that they meet each other posteriorly.

The walls of the reservoirs are extremely thin and are more or less smooth when distended with secretion but much wrinkled when empty. The structure of the walls of the central and the lateral reservoirs is identical. They are composed of a uniform layer of thin chitin, lined externally by a layer of flattened epithelium. In some places the cell boundaries in the epithelial lining are not clearly seen and the cellular nature of the epithelium in such places is only recognizable through the presence of the nuclei. There appear to be no muscles in the walls of the reservoirs and the wrinkled appearance of the walls must therefore be due to the contraction of the chitinous intima of the reservoirs. Murray (1914) in his figure (31) of the stink-apparatus of the bed-bug shows two regions in the anterior wall of the central reservoir, one of large and the other of small cells. In none of my preparations of the reservoirs have I seen any other cell in the anterior wall than the flat epithelial cells which correspond to Murray's small cells. The large cells described by him are undoubtedly fat-body cells which are found closely applied to the anterior wall of the central reservoir. In the same figure he shows two thick layers, an outer (light) and an inner (dark), in part of the wall of the lateral reservoirs. In sections the walls of the reservoirs have a fairly thick appearance but this is only due to their being much contracted and wrinkled. The chitinous lining (which stains violet with Mann's solution) can easily be seen as a continuous layer going in and out of these undulations. Except in the region of the kidney-shaped thickening in the posterior wall of the central reservoir, there is only one type of large flat cell forming the epithelial lining of the three reservoirs. The structure of the walls of these sacs clearly shows that they are not of a glandular nature and can therefore only be considered as reservoirs.

Laterally the central reservoir narrows down and is continued on each side as a tubular *efferent duct* (*e.d.*). The chitinous lining of the posterior wall of this tube shows slight projections into the lumen, while that of the anterior wall is smooth and thickened. Each of these two ducts opens to the exterior by a small crescent-shaped aperture (*O*) lying near the ventral border of the metapleura. I have been unable to find any median ventral opening on the metasternum, as described by Landois and Murray. In some specimens the

central reservoir shows a narrow downward projection but it has no external opening. When the stink-apparatus is dissected out the reservoirs retain their secretion and remain fully distended, even though the ventral body-wall of the bug is completely removed from it. It is obvious that the secretion must have escaped from the reservoirs if there had been any ventral opening of the central reservoir on the metasternum.

In other families of the Hemiptera-Heteroptera not only does the form of the reservoirs differ in the adult bug, according to the family, but the number

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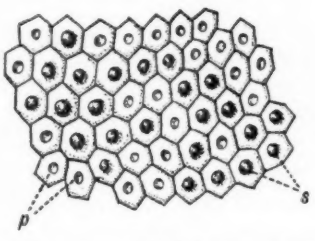

Fig. 5. Surface view of a portion of the kidney-shaped organ. ×1240.

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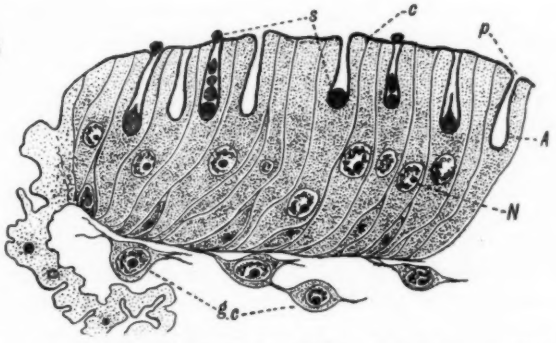

Fig. 6. A part of a section through the kidney-shaped organ. ×1240.

A. Ampullae. *c*. Chitinous lining. *g.c*. Ganglion cells. *N*. Nucleus. *p*. Pores of the ampullae. *s*. Globules of coagulated secretion.

and position of the external openings also varies. Bergroth (1906) in *Gerridae* and Krüger (1909) in *Nepidae* and *Notonectidae* and a few other aquatic families, have recorded only a single median opening on the metasternum, while Muir (1907) describes one aperture on each side on the metathorax of *Tessaratoma* (*Pentatomidae*) and Dufour (1833) found the same number in a number of families. But in no case have both lateral and median ventral openings been found occurring together in the same bug, as has been erroneously described by Murray in *Cimex lectularius*.

The Median Kidney-shaped Organ. This organ (*K*, Fig. 1) forms a part of the posterior wall of the central reservoir. It is composed of a layer of elongated conical cells (Fig. 6), the wider or inner ends of which form the lumen of the reservoir, while the narrow or outer ends are directed towards the perivisceral or body cavity of the insect. In each of these cells a large nucleus (*N*) lies in the middle or near the narrow end. The inner surface of these cells is lined with chitin (*c*), which is in continuation with that of the rest of the reservoir. This chitinous layer invaginates into each of the cells of the kidney-shaped organ and there forms characteristic goblet-shaped depressions (*A*) in the outer portion. These depressions have been referred to by Murray as *ampullae*.

Murray describes these ampullae as solid club-shaped bodies with the handle of the club projecting slightly above the surface of the cells into the lumen. Sagittal and frontal sections of this organ show small globules (*s*) projecting from the openings (*p*) of the ampullae, but these bodies are found only in those reservoirs, which contain secretion of the gland, and always stain differently from the chitin. It is therefore almost certain that the bodies, seen by Murray projecting from the ampullae, were droplets of secretion coagulated by the fixative.

In close apposition to the posterior side of this kidney-shaped organ there rests a network of small ganglionic cells (*g.c.*) which is connected with the sympathetic nervous system lying in the region of the thoracic ganglion. I am unable, however, to confirm Murray's view as to the existence of direct nerve-communication between the thoracic ganglion and the kidney-shaped organ.

The function of this organ will be dealt with in the last section of this paper.

Development of the Stink-apparatus.

The metathoracic stink-apparatus as seen in the adults of both sexes is not found fully developed in any of the five larval stages. It makes its first appearance in the fifth larval stage (or nymph) four to six days after the fourth larval moult. It appears as two lateral invaginations of the epithelial lining of the body-wall in the region of the metathorax. These invaginations grow inwards, gradually becoming larger and bilobed at their distal ends. From the anterior, larger lobe, a solid mass of cells grows out, remaining attached to it only by a very narrow neck—the future main duct of the gland. The cells of this body differentiate into the unicellular glands with their intra-protoplasmic ductules and the small epithelial cells which secrete the chitinous lining of the collecting duct, thus forming one complete gland on each side. A number of cells in the posterior wall of the anterior lobe become very much elongated and these later form the kidney-shaped organ. At the inner ends of each of these elongated cells a small vacuole appears which increases in length and finally bursts into the cavity of the invagination, forming the ampullae (of the kidney-shaped organ). The rest of the cells

forming the walls of both the anterior and the posterior lobes become very flat.

The neck of the original invagination becomes very thin and finally breaks off from the epithelial lining of the body-wall. At this stage there is recognizable on each side of the body a gland and a bilobed sac, the anterior lobe of which has a rounded patch of elongated cells.

The posterior lobes become elongated and form the lateral reservoirs. The anterior ones grow inwards and, meeting in the median line, fuse into one central chamber—the central reservoir of the adult.

Previous to the last moult an invagination is formed beneath the nymphal cuticle in the region of the metapleura, and this invagination meets the outwardly growing central chamber and forms the opening of the stink-apparatus in the adult. If the stink-apparatus is dissected out from the nymph at this stage, it is seen to be complete in all details except that, instead of having one median kidney-shaped organ, there are two small rounded ones placed laterally. The two portions grow inwards towards each other, meeting in the middle line to form a more or less dumb-bell shaped structure, which later on becomes the kidney-shaped organ of the stink-apparatus.

Stink Organs of the Larval Forms.

During the five larval stages, *Cimex lectularius* is provided with stink organs which differ both in form and position from those found in the adult. They are three is number and, like those of other larvae of Hemiptera-Heteroptera, are found in the abdomen along the medio-dorsal line. They are very simple in structure, the gland cells being arranged in a single layer, in the form of a semicircular sac lined by a thin layer of chitin. Each of these sacs lies closely applied to the body-wall, with its curved side directed anteriorly. The sac opens externally by a pair of slit-like apertures, one on each side, at the posterior border of the segment to which it belongs.

The stink organs of the larvae of *Cimex* were first described by Künckel d'Herculais (1886) as composed of three more or less semicircular sac-like glands and according to him they are situated one in each of the first three abdominal segments. Hase (1917) in his figures 17—21, 27 and 28, shows three glands as lying in the third, fourth and fifth abdominal segments and not in the first three as stated by Künckel d'Herculais. This discrepancy can easily be explained, for Christopher and Cragg (1921) have shown that the first completely chitinised tergum in the abdominal region of the larva belongs to the third abdominal segment and not the first. It is under this tergum that the first of the three stink-glands lies.

The number of stink-glands is not constant in the larvae of Heteroptera. Sharp (1918) in his description of the internal anatomy of Hemiptera says that Heteropterous larvae have two glands, situated dorsally and opening on two of the dorsal plates of the abdomen, but does not mention on which segments they open.

Function of the Stink-apparatus.

Bordas (1898, 1899) and Dierckx (1899, 1901) have described anal or pygidial glands at the posterior end of a large number of Coleoptera with histological structure (specially in the genus *Claenius* and *Clivina*) similar to that of the stink-gland of *Cimex*. Like the stink-glands of the bed-bug, in all these cases the insects secrete a malodorous, yellowish, volatile fluid. These authors agree that the glands are organs of defence. Similar glands have been described by Bordas (1901) in the male Cockroach (*Periplaneta americana* and *P. orientalis* L.), but their presence only in the male and their connection with the genital organs indicates that their function may be related to the sexual activity of the insect. Bordas on the other hand considers them as organs of defence only. In the Coleoptera the reservoir is provided with a muscular coat and the insect can therefore eject the stinking fluid at will. But in *Cimex*, I have not been able to find any muscles in connection with the reservoir and it seems likely that the fluid oozes out of the external apertures at all times. It is probable that, when the bug is at rest, the coxae of the second pair of legs serve to block the opening, at least partially; but that, when the bug is disturbed and begins to move about, the obstruction to the outflow of the offensive odour is removed and it is therefore more pronounced.

Stink organs as organs of defence are of common occurrence among insects and on comparing the stink-apparatus of *Cimex* with those of others (especially with those of the larger Heteroptera) it appears that in this insect too the function is the same.

Landois thinks that the secretion of the gland is more abundant in summer because the vitality of the bug increases with the heat. Murray in his description says that "there would appear to be some evidence to show that cold reduces the size of the glands (by which he means the lateral reservoirs) while heat increases it but hardly enough to dogmatise on the matter." My own observations, extending through the winter and the greater part of the summer, have led me to no definite conclusion as to the cause of the variation in the amount of secretion. The majority of bugs, irrespective of sexual maturity, showed in winter fully distended reservoirs, while in summer quite a large number had the reservoirs much contracted. In some specimens the lateral reservoirs appeared only as two small protrusions on the posterior wall of the central chamber. The secretory activity of the gland does not seem to depend even on the age of the bug, since young adults often show the reservoirs much more distended than those of the older bugs.

As to the function of the kidney-shaped organ within the central reservoir, Murray suggests that it is an olfactory organ and in concluding his reference to it, adds, "In his (*sic*) series of sections, some show absolutely no trace of it, and this is not due to tearing of the sections, for even if torn, the organ can easily be recognised although its position may be changed." He therefore thinks that this organ may become hypertrophied at times of sexual activity

and at other times may be so small as to be practically invisible. My personal observations on the contrary show that this organ is common to *all adult* bed-bugs, irrespective of their sexual activity, their age, or the time of year, and without any material difference of size. Very little can be said as to the function of this organ, but the fact that it is confined to the adult indicates that it has some connection with the sexual activity of the bug. Its structure suggests either a secretory or a sensory function. If it be the latter, it is possible that the secretion of stink-glands in the two sexes are in some way slightly different from one another and that this peculiar kidney-shaped organ has the power to distinguish them. In this case the stink-apparatus in *Cimex lectularius* would serve two functions: (1) a *defensive*, and (2) a *sexual* one.

Summary.

The stink organs are different in structure and position in the adult and the larval stages.

The stink organs of the adult are composed of (*a*) a pair of *glands*, (*b*) a *central reservoir* with two backwardly-directed *lateral reservoirs*, (*c*) a median *kidney-shaped organ* in the central reservoir, and (*d*) a pair of lateral external openings.

Each gland is a multicellular compound structure, opening into the central reservoir. It is described for the first time in this paper.

The whole of the stink organ of the adult is formed in the last larval stage from a pair of invaginations of the epithelial lining of the body-wall.

In the larval stages the stink organs are simple glands, situated dorsally in abdominal segments III to V.

The function of the stink organs is defensive and in the adult perhaps also sexual.

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ON SOME NEW CEPHALINE GREGARINES[1].

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(With 23 Text-figures.)

Leidyana xylocopae sp. nov.

(Figs. 1–8.)

Host: *Xylocopa aestuans* (Linn.). Locality: Lahore, India.

The gregarine described below has been found abundantly in the alimentary canal of many specimens of the carpenter bee, *Xylocopa aestuans* (Linn.), and is the first to be described from any Hymenopteran host. Keilin (1918), in describing *Leidyana tinei* in a Lepidopterous larva, remarked that no gregarines had been found till then in the two large orders of holometabolic insects: Lepidoptera and Hymenoptera. He further observed as follows: "The fact that gregarines have not been previously recorded in these two orders cannot be considered as due to lack of observation, since large numbers of these insects have been dissected for many different purposes. Possibly we can account for the infrequency of their occurrence because of the habits of the larvae of Lepidoptera and Hymenoptera. These are often parasites in other Arthropods (Hymenoptera) or they are gallicolous (Hymenoptera) or phytophagous (Lepidoptera and Hymenoptera) or they live in nests and cells (Hymenoptera)." We have examined about 200 specimens of *Xylocopa* and found them practically always infected.

These carpenter bees were found in abundance on rose bushes in April (1922). A month later they became rather scarce and extremely difficult to procure. They were then occasionally met with on the flowers of *Calotropis gigantea* growing near the banks of the river Ravi, and still later in May on certain plants of the order Compositae on the banks of a canal.

The parasite occurs in large numbers throughout the length of the alimentary canal in every host. The gut appears tightly packed with the parasites, and yet the host does not seem to suffer any inconvenience from their presence. The gut-contents consist also of pollen grains and other food substances ingested by the bee.

[1] This paper is based on the work carried on by Mr Setna in 1922, under my direction. Mr Setna made all the preparations whilst I have re-examined them, revised and partly re-written the paper, made all the measurements and re-drawn the figures.—B. L. B.

Our thanks are due to Dr Annandale, Director, Zoological Survey of India, for the loan of some works of reference from the Library of the Indian Museum, Calcutta.

The gregarine is of chalky white colour and is filled with spherical food granules, which sometimes occur in such numbers as to mask the nucleus. These granules are soluble in strong but insoluble in dilute mineral acids; insoluble in alcohol, and are coloured brown on the addition of iodine, thus showing that they are composed of paraglycogen.

The parasite exhibits extremely slow movements which are noticeable only under high magnification, but its body is extremely deformable especially in the protomerite region, the protoplasm of which may suddenly flow forward or backward. The gregarine never moves backward.

Only the trophozoite (cephalont) and sporont stages have been encountered.

Cephalont. The epimerite is a large, simple, and sessile knob, narrowing but slightly near the base. Only a few trophozoites are found, these being usually attached to the cells of the intestine. Measurements in microns of a fairly large cephalont (Fig. 1) are: total length (TL) 90, length of epimerite 13, protomerite (LP) 9, deutomerite 68, width of protomerite (WP) 13, deutomerite (WD) 20. Ratio of LP : TL :: 1 : 10. Ratio of WP : WD :: 1 : 1·4.

The *sporonts* are always solitary; they vary in form and size; the smallest measure 23μ, the largest 174μ in length. The following table gives the measurements in microns:

Sporont length	56	64	76	92	140	174
Protomerite length ...	12	13	13	13	28	28
Deutomerite length ...	44	51	63	79	112	146
Protomerite width ...	12	13	27	13	22	36
Deutomerite width ...	18	18	46	24	30	51
Ratio of protomerite length to total length	1 : 4·6	1 : 4·9	1 : 5·8	1 : 7	1 : 5	1 : 6·2
Protomerite width to deutomerite width	1 : 1·5	1 : 1·3	1 : 1·7	1 : 1·8	1 : 1·3	1 : 1·4
Diameter of nucleus	12	11	20	16	20	18

Usually the outline of the body is elongated and cylindrical, rounded anteriorly and slightly tapering posteriorly. The full-grown sporont and young trophozoite differ in size and the endoplasm of the sporont is more coarsely granular, thereby rendering the organism almost opaque and difficult to stain. The structure of the gregarine could be satisfactorily studied in sections of the host's gut and in the free individuals.

The protomerite is rounded or dome-shaped and there is a deep constriction at the septum. The deutomerite is elongated, slightly broader in the middle and narrowing posteriorly. The posterior end of the body is always rounded. Sometimes thick-set forms (Fig. 4) are met with in which the body does not narrow posteriorly but is broadly rounded. The nucleus varies in position in the deutomerite. Stained with iron haematoxylin the nucleus has the form of a large vesicle bounded by a sharply-defined nuclear membrane; it contains a large spherical karyosome, placed centrally or eccentrically and surrounded by chromatin granules. The karyosome often appears vacuolated. The nucleus in one of the largest specimens measured 24μ and the karyosome

8μ in diameter. The nuclear membrane and karyosome stained deeply with iron haematoxylin while the rest of the nuclear substance takes a bluish tinge.

Although the gregarine was studied over a period of some months and many individuals were examined, neither cysts nor spores were encountered. Probably conjugation and spore-formation occur within the body of the larvae of *Xylocopa*. In the absence of information about the dehiscence of the cysts and the form of the spores, it is impossible to establish the exact systematic position of this new parasite. But from the possession of a simple, symmetrical epimerite and the solitary character of the sporonts, it seems reasonably certain that it belongs to the genus *Leidyana*. Although in the proportion of its parts, and the form of the protomerite it has some resemblance with *L. gryllorum* (Cuénot) Watson and *L. tinei* Keilin, yet it differs from these in the form of its epimerite, its occurrence in a widely different host belonging to a distinct order of insects. This justifies the conclusion that it is a new species.

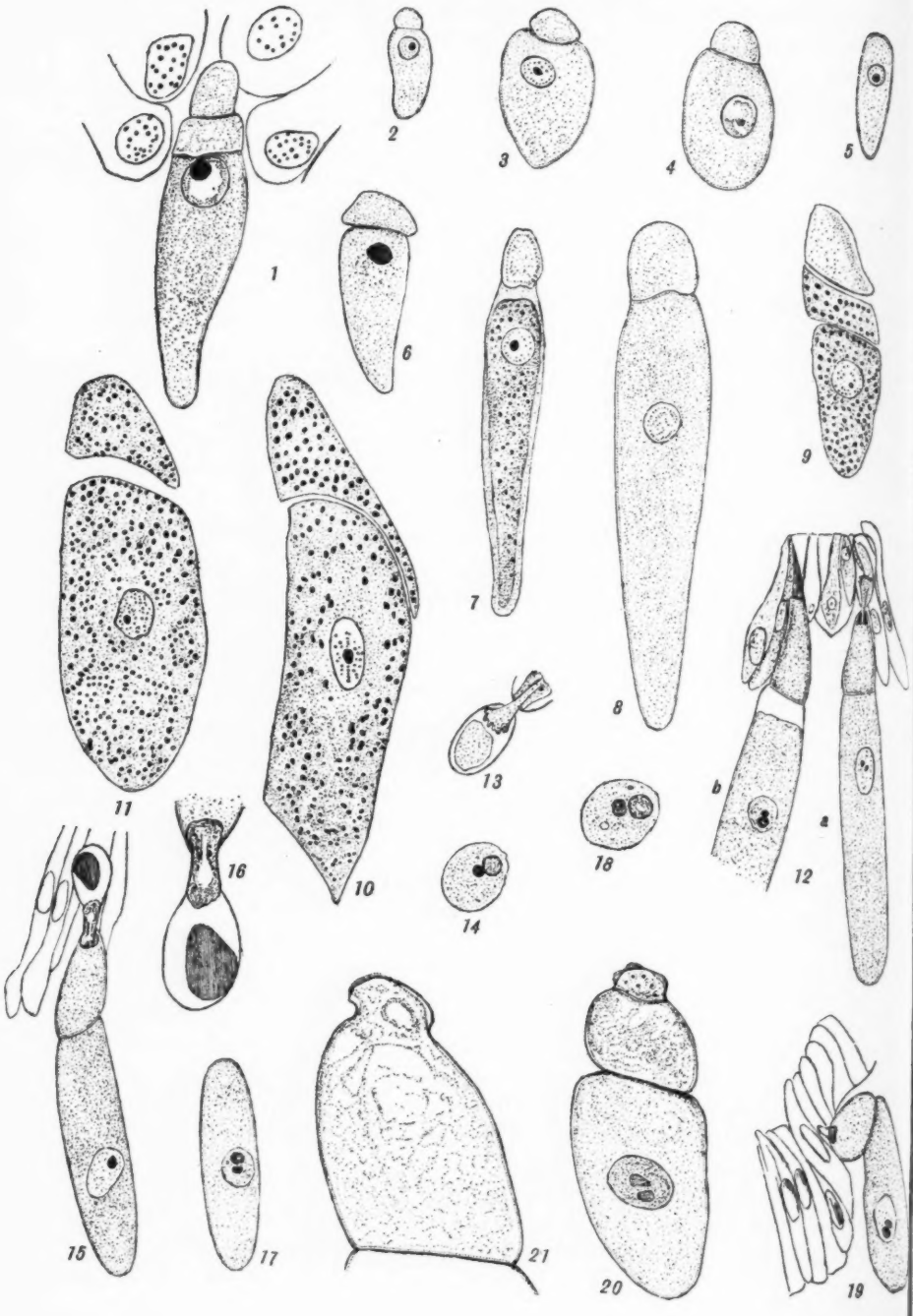
Leidyana leptoplanae sp. nov.

(Figs. 9–11.)

The parasite was found in *Leptoplana* sp. in 1922, and a short note regarding it was published in *Nature*. Only a single specimen out of a number in the same tube received from the Marine Biological Laboratory, Plymouth, was found to be infected. It seems to be a rather rare parasite of *Leptoplana* for it has not been described hitherto although the host is a common laboratory type frequently sectioned for examination by the students everywhere, whilst the parasite is so large and possesses such a characteristic structure that it could not easily have been missed. Reference to lists of sporozoan parasites and their hosts (Minchin (1903), Watson (1916) and Watson Kamm (1922)) shows that no gregarine has been described from *Leptoplana* before, and that only two cephaline gregarines have been recorded previously from Platyhelminths, these being: *Gregarina* sp. (Hallez) in *Dendrocoelum lacteum* and a gregarine form, found by Wellmer in *Polyporus sulphureus*.

In sections of the infected worm (*Leptoplana*) a number of the gregarines were found occupying cavities in the parenchyma of the host's body. The sporonts were all solitary, and measured from 56 to 173μ in length. Measurements of a number of individuals are given below in microns:

Cephalont length	...	94	—	—	—
Sporont length ...	...	—	56	154	173
Epimerite length	...	25	—	—	—
Protomerite length	...	11	20	30	33
Deutomerite length	...	58	36	124	135
Protomerite width	...	45	20	70	60
Deutomerite width	...	43	33	67	82
Ratio of LP : TL	...	1 : 5·2	1 : 2·8	1 5·1	1 : 5·2
Ratio of WP : WD	...	1 : 1	1 : 1·6	1 : 1	1 : 1·3
Diameter of nucleus	...	22	—	22	22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Among the specimens there is a single cephalont (Fig. 9) the measurements of which are given in the table p. 281. The epimerite is large, broadly conical in outline, finely granular and free from paramylum granules. The protomerite is flattened like a disc between the epimerite and the deutomerite. The deutomerite is widest at the shoulder and gradually narrows to the posterior end. Both the protomerite and the deutomerite are full of spherical granules of paramylum, which stain black with iron haematoxylin. The full-grown sporont attains as much as 173μ in length and 82μ in width, the ratios being as stated above. The protomerite in the sporonts is broadly conical, widest at the base, and fitting like a cap over the anterior end of the deutomerite, from which it appears to be separated by a lesser or greater clear area or space (see Figs. 10 and 11) corresponding to the septum. In the specimen shown in Fig. 11, this area, which is structureless, measures 5μ along the length of the gregarine, the latter's total length in microns being P 33μ + 5μ + D 135μ = total 173μ. The deutomerite is elongated, cylindrical, widest at the anterior end, and broad and flattened or broadly rounded posteriorly. The nucleus, which is spherical or oval (*ca.* 22μ), is placed in the anterior half of the deutomerite; it has a fine nuclear membrane and deeply-stained chromatin particles; the karyosome is single, spherical, and placed centrally or eccentrically.

No cysts or spores have been encountered. Owing to the possession of a simple epimerite and to the solitary character of the sporonts the parasite is referred to the family Gregarinidae, and provisionally placed in the genus

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EXPLANATION OF TEXT-FIGURES 1—21.

All the figures were drawn from permanent preparations.

Figs. 1, 5, 12–19 are from sections stained with borax-carmine; Figs. 9–11 from sections stained with Delafield's haematoxylin; and Figs. 6–8, and 20–23, from smears stained with iron haematoxylin.

Leidyana xylocopae n. sp.

Fig. 1. Cephalont, with the epimerite sticking among the epithelial cells of the host.
Figs. 2, 3, and 4. Sporonts lying free in the gut.
Fig. 5. Deutomerite lying free.
Fig. 6. Sporont lying free, nucleus not differentiated.
Figs. 7 and 8. Full grown sporonts.

Leidyana leptoplanae n. sp.

Fig. 9. Cephalont.
Figs. 10 and 11. Sporonts.

Caulocephalus crenata n. g., n. sp.

Figs. 12, 15, and 19. Cephalonts attached to the host's epithelial cells.
Fig. 13. Epimerite of 12 *a*, more highly magnified.
Fig. 14. Nucleus of 12 *b*.
Fig. 16. Epimerite of 15.
Fig. 17. Deutomerite lying free in the gut-lumen.
Fig. 18. Nucleus of 17.
Fig. 20. Sporonts showing crenated epimerite and nuclear structure.
Fig. 21. Epimerite and protomerite of full-grown sporont.

Leidyana. The epimerite however is not like a sessile globe as in the previously recorded species of *Leidyana*.

Caulocephalus crenata g. et sp. nov.

(Figs. 12–23).

Host: adult specimens of *Aulacophora foveicollis* Kust. (= *A. abdominalis* G. et H.). Locality: Lahore and Kalka, Punjab, India, in April–September, 1922.

This parasite is found in very large numbers, throughout the entire length of the alimentary canal. A very high percentage of the beetles examined were found to be heavily infected. As many as 100 parasites could be counted in a single smear, that is to say, a single host may easily harbour several hundreds of these parasites.

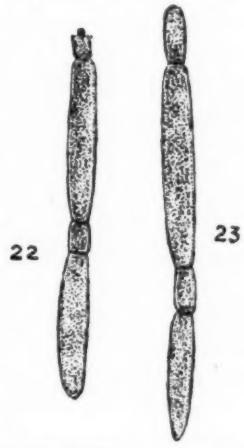
When alive, the gregarines generally appeared densely granular and opaque, the nucleus being invisible. But by starving the host for a number of days, the dark granules in the endoplasm of the parasite were found to disappear.

Cephalonts. The epimerite is usually dilated anteriorly like a cauliflower and narrower basally where it arises from the protomerite (Figs. 12, 13, 15 and 16). Its surface is not smooth, but crenate. In sections of the host's intestine the epimerite is found inserted within the cell of the host. The protoplasmic substance of the epimerite appears densely granular, but there is a central hollow portion, which extends like a tube into the anterior part of the protomerite, where it is surrounded by the same densely granular substance as in the epimerite. This substance is more deeply stained than the rest of the protomerite, and forms a sort of pedestal for the tubular stalk of the epimerite. In other specimens the epimerite forms a compact, oval knob-like projection with crenated surface; it may or may not exhibit a central cavity (Figs. 20 and 21). Where the central cavity is absent the characteristic specialised zone (*v. infra*) in the protomerite may also be sometimes absent. Even fully developed free individuals sometimes retain a small globular and sessile knob at the anterior end but the surface of the epimerite is always crenated. The protomerite is elongated and conical and usually $1\frac{1}{2}$ times as long as wide; it is usually widest about the middle, narrowing slightly towards the base; its anterior margin is rounded or slightly concave. There is a characteristic specialised zone at the anterior end of the protomerite, which is followed by a clear zone crossed by protoplasmic strands. There is a distinct constriction at the septum. The deutomerite is cylindrical, $1\frac{1}{2}$ to 3 times as long as broad; it is widest at its middle and gradually narrows towards the rounded posterior end. The nucleus is large and spherical or somewhat oval and usually placed about the middle or a little in front of it; it has a distinct nuclear membrane and encloses two karyosomes, the larger vacuolated, the smaller more compact (Figs. 12 *b*, 14, 17, 18). In some cases however there is a single karyosome, which is placed eccentrically.

The following table gives the measurements in microns:

Total length	40	52	60	77	124	135	142
Epimerite length ...	7	7	6	7	7	7	7
Protomerite length ...	10	15	18	22	36	38	37
Deutomerite length ...	23	30	36	48	81	90	98
Protomerite width ...	17	18	18	21	27	25	26
Deutomerite width ...	21	22	22	28	43	34	34
Ratio:							
Protomerite length to total length	1 : 4	1 : 3·4	1 : 3·3	1 : 3·5	1 : 4	1 : 3·5	1 : 3·8
Protomerite width to width of deutomerite	1 : 1·2	1 : 1·2	1 : 1·2	1 : 1·3	1 : 1·5	1 : 1·3	1 : 1·3
Diameter of nucleus ...	9	10	11	12	16	15	16
Number of karyosomes ...	one	two	two	one	two	one	one

Sporonts. The epimerite persists in well-developed free individuals, though it undergoes a good deal of alteration in its appearance as previously indicated. Owing to the retention of the epimerite, it is difficult to say when the organism may be said to have entered on its sporont phase. However, judging from a certain number of individuals deprived of their epimerite, it was noticed that although cephalonts may be found to attain the size of a sporont, in the latter the protomerite will be found to be considerably reduced in proportion to the total length of the body. Thus the measurements in a typical case were: total length 134, length of protomerite 22, deutomerite 112. Width of protomerite 22, deutomerite 37. Ratio of LP : TL :: 1 : 6. Ratio of WP : WD :: 1 : 1·6.



Caulocephalus crenatus n. sp.
Fig. 22. An association, with primite showing a persistent epimerite.
Fig. 23. A normal association.

Associations. The sporonts are bi-associative. The sporonts forming associations are found to be much more elongated than the cephalonts (Figs. 22 and 23). In one instance (Fig. 22), the primite still possessed a small knob-like epimerite. This strange condition has been previously found in *Gregarina rigida* (Hall) Ellis, *vide* Watson (1916, Fig. 337). The satellite has a broad truncated conical protomerite and the ends of the primite and satellite are joined by a straight line, there being no device or modification for interlocking. The usual measurements in microns follow:

Total length association	363	445
Primite:		
Total length	198	225
Epimerite length	—	7
Protomerite length	42	38
Deutomerite length	156	180
Protomerite width	30	30
Deutomerite width	40	34

Ratio:		
Protomerite length to total length	1 : 4·7	1 : 5·9
Protomerite width to width of deutomerite ...	1 : 1·3	1 : 1·1
Satellite:		
Total length	165	220
Protomerite length	38	45
Deutomerite length	127	175
Protomerite width	34	34
Deutomerite width	38	42
Ratio:		
Protomerite length to total length	1 : 4·3	1 : 4·9
Protomerite width to width of deutomerite ...	1 : 1·1	1 : 1·2

In two rare instances two satellites were found attached to the hinder end of a primite, as previously recorded by Léger for *Hirmocystis polymorpha* (*vide* Minchin, 1903, Fig. 24*b*), but unlike that case, the satellites had not lost their protomerite in our specimens. In this respect also, these rare associations resembled the unique association of three sporonts previously found in *G. rigida* (*vide* Watson, 1916, Fig. 336).

Cysts and spores. We have not observed the process of cyst and spore-formation, but there are some cysts in our smears. These are spherical and are ruptured, containing some spores, a considerable number of which were scattered outside the cysts. The latter measure about 90μ in diameter. The spores are ovoid or nearly spherical, and 12μ in diameter.

Systematic position. The epimerite of the trophozoites attached to the host's cells, suggests that *Caulocephalus crenata* possesses affinity with the family Actinocephalidae, but it cannot be placed in that family as the sporonts are bi-associative. The epimerite also, though presenting a crenated surface, cannot be regarded as complex; it varies in appearance and ultimately becomes simple and symmetrical. Therefore the organism belongs to the Gregarinidae although it cannot be referred to any known genus of that family. The protomerite resembles that of *Pyxinoides balani* and *P. cthamali* (*vide* Trégouboff, 1912), but the epimerite differs distinctly from that of *Pyxinoides* which is described as a style dilated in the middle. It is therefore proposed to create for our parasite a new genus: *Caulocephalus* (the epimerite resembling, in its more characteristic phases, a cauliflower).

Diagnosis of the genus. Bi-associative, satellite with a septum. Epimerite dilated anteriorly like a cauliflower and narrower at the base. Protomerite with a characteristic specialised zone anteriorly. Cysts dehiscing by simple rupture. Spores ovoid or spherical.

Confinement to certain localities. An interesting observation on the parasites being confined to hosts in certain localities may here be recorded. At Lahore, in April to July, 1922, the host beetles were found to be heavily parasitised, nine out of every ten beetles harbouring *C. crenata*. From July to September, the same species of beetles were found at Kasauli (over 6000 feet), but over a hundred were examined and not one was found infected. Again at Kalka (9 miles from Kasauli) some of the beetles were examined and were found

to be parasitised as at Lahore. A somewhat similar observation was made by Watson in the case of some Orthoptera. The explanation she suggests for this phenomenon is that the spores, having once become established in restricted areas, have not yet found the means of being widely scattered.

Gregarina oviceps Diesing.

(SYN. *Gregarina Achetae abbreviatae* Leidy; *G. achetae abbreviatae* Labbé.)

Taken in Lahore, India. HOST: *Gryllus* sp. HABITAT: gizzard and mid-gut.

A number of gregarines have been previously described from different species of *Gryllus*. We have been unable to determine the specific name of our specimens of *Gryllus*. The insect harbours in its alimentary canal at least two distinct species, which whilst differing in minor respects, may be referred to *Gregarina oviceps* Diesing and *Leidyana gryllorum* (Cuénot) Watson. A small proportion only of insects examined were found to be infected, and parasites were confined to the gizzard and the mid-gut.

The parasites referred to *G. oviceps* are bi-associative and distinctly obese. The essential measurements of free individuals are as follows (in microns):

Cephalont length	160	—	—	—	—
Sporont length	—	92	142	195	206
Epimerite length	20	—	—	—	—
Protomerite length	28	22	37	38	34
Deutomerite length	112	70	105	157	172
Protomerite width	49	22	58	67	56
Deutomerite width	65	37	101	119	101
Ratio:					
Protomerite length to total length	1 : 5·7	1 : 4·1	1 : 3·8	1 : 5·1	1 : 6
Protomerite width to width of deutomerite	1 : 1·3	1 : 1·2	1 : 1·7	1 : 1·7	1 : 1·8

Similar measurements of a few associations are as follows:

Total length association	720	672
Primite:	90	65
Protomerite length	270	270
Deutomerite length	157	157
Protomerite width	157	157
Ratio:		
Protomerite length to total length	1 : 4	1 : 5·1
Protomerite width to width of deutomerite	1 : 1	1 : 1
Satellite:		
Protomerite length	68	55
Deutomerite length	292	292
Protomerite width	135	135
Deutomerite width	146	146
Ratio:		
Protomerite length to total length	1 : 5·3	1 : 6·3
Protomerite width to width of deutomerite	1 : 1	1 : 1

On comparing the foregoing measurements with the synopsis of the characters of the species, we find that the sporonts examined by us are shorter and narrower, that the ratio of the length of the protomerite to the total length of the primite is as 1 : 4 or even 1 : 5·3 instead of 1 : 3 as given by Watson, but that the width of the protomerite to the width of deutomerite is practically the

same. The protomerite is hemispherical to subglobose and its width is twice its length or even slightly more. The deutomerite is stout, and may be nearly as wide as long, or up to twice as long as wide. The deutomerite is widest at the shoulder where it is only as wide as the protomerite. The posterior end is truncate. The endocyte is denser in the deutomerite than in the protomerite, and is distinctly marked off from the sarcocyte.

According to Watson the epimerite in this species is unknown, and the nucleus has not been seen because it is invisible *in vivo*. We are able to give information on these points. The epimerite is simple and elongated like a nipple; the nucleus in the full-grown sporonts is oval, has a fine nuclear membrane, and contains many fine chromatin granules but no karyosome.

Leidyana gryllorum (Cuénot) Watson.

This gregarine occurs with *G. oviceps* in the same host. Their characters are as follows: sporonts solitary and cylindrical. Protomerite subspherical with deep constriction at septum. Deutomerite cylindrical, generally conical at the end. Epimerite globular and sessile. Nucleus spherical, generally with three karyosomes and numerous fine chromatin particles.

Watson has separated this species from *L. erratica* on the basis of two characters, viz. (1) that the protomerite is conical at the apex (never rounded) in *L. erratica* and broadly rounded—subspherical—in *L. gryllorum*, and (2) the constriction at the septum is considerably deeper in *L. gryllorum*. Further, as regards the deutomerite, we find it stated that it is conical at the end in *L. gryllorum*, sometimes tapering but always rounded at the end in *L. erratica*. Otherwise the "size of the two is nearly the same, ratios of various parts not radically different and shape of the deutomerite quite similar."

Judging from our specimens, we are inclined to cast doubt that *L. erratica* and *L. gryllorum* are distinct species. Specimens having a broadly rounded protomerite may be rounded posteriorly, and conversely a specimen which might be referred to *L. gryllorum* (by virtue of a deep constriction at the septum and a conical posterior end) may possess a protomerite which is conical anteriorly. Several specimens show intermediate conditions in all these respects.

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SOME PARASITIC COPEPODA FROM ICELAND.

WITH AN ACCOUNT OF *PENICULUS CLAVATUS*, THE CONJUNCTIVE TUBES OF *CHONDRACANTHUS NODOSUS* AND THE MALES OF *CLAVELLA DUBIA*.

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AND
MICHAEL G. L. PERKINS, B.A. (Cantab.), F.E.S.

(With 8 Text-figures.)

The following is a list of parasitic Copepoda collected by L. Harrison Matthews during a fortnight's visit to Iceland in the first half of July 1922, some thousands of fish being examined at the numbered stations (St.), viz.: (1) Veetmannaeyjav (Westmanna Isles) E. 10 m.; (2) Storknaes; (3) Portland (Iceland).

1. CALIGUS CURTUS Müller.
   (*a*) 6, 5, and 3 specimens respectively from *Gadus virens* (St. 1); (*b*) 1 ♀ which had just changed its skin, from *Gadus morrhua* (St. 1).
2. CALIGUS RAPAX M. Edwards.
   (*a*) 4 ♀♀ with Vorticellid and Rotifer (?) Epizoa from *Hippoglossus vulgaris*; (*b*) 19 ♀♀ (and the Amphipod *Hyperia*) from mouth and pharynx of *Gadus virens* (St. 1); (*c*) 3 ♀♀ heavily infested with a Vorticellid from *Gadus morrhua* (St. 1).
3. LEPEOPHTHEIRUS HIPPOGLOSSI (Krøyer).
   6 ♀♀ (St. 2) and 1 ♂ 2 ♀♀ from *Hippoglossus vulgaris*.
4. HATSCHEKIA HIPPOGLOSSI (Krøyer).
   6 ♀♀ from *Hippoglossus vulgaris*.
5. LERNAEA BRANCHIALIS Linnaeus.
   6 ♀♀ from the gills of *Gadus morrhua* (St. 3).
6. PENICULUS CLAVATUS (Müller).

This species has hitherto been imperfectly described from Greenland only. The following description relates to:

Material. 1 ♀ found (4. vii. 1922) attached to a ray of the dorsal fin of *Sebastes norvegicus* (St. 1).

Female. The general form is depicted in Fig. 1. The total length of the preserved specimen is 24·03 mm., of which the attaching organ occupies 0·13 mm., the head 0·3 mm., the neck 0·7 mm., the trunk 5·4 mm., and the egg-strings which are extraordinarily long 17·5 mm., each containing about 330 disc-like ova in a single series.

The *cephalothorax* consists of a stoutly pyriform anterior end or head bearing the appendages, and an elongated, slender neck connecting head and trunk.

The *head* bears anteriorly and dorsally a frontal organ of attachment which differs from that of other Lernaeidae. It is strongly chitinised and resembles in form a short-armed tuning-fork, the apex of each arm ending in a claw-like appendage embedded in wound tissue, and was so closely adpressed to the

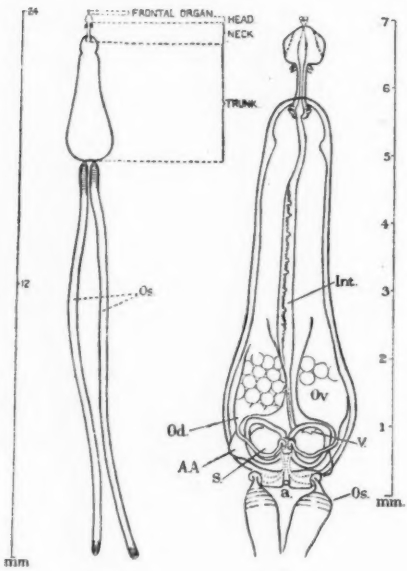

Fig. 1 Fig. 2

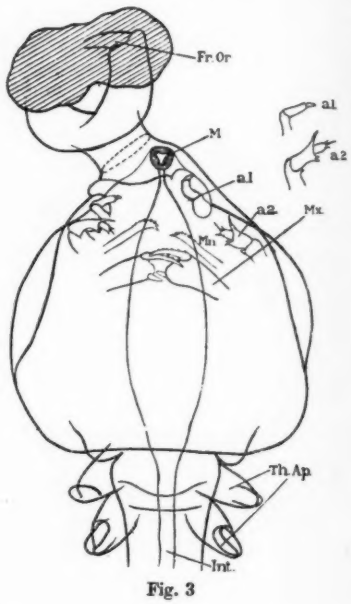

Fig. 3

Fig. 1. *Peniculus clavatus*, dorsal aspect. *Os.* ovisacs.

Fig. 2. *Peniculus clavatus*, dorsal aspect. *Int.* intestine; *a.* anus; *Od.* oviduct; *Ov.* ovary; *V.* vulvae; *S.* spermathecae; *Os.* ovisacs; *A.A.* abdominal appendages.

Fig. 3. *Peniculus clavatus*, enlarged view of the anterior end, mounted in Farrant's medium, dorsal aspect, but viewed as a transparency. *Fr.Or.* frontal organ of attachment; *M.* mouth; *a.*1. antennules; *a.*2. antennae; *Th.Ap.* thoracic appendages; *Int.* alimentary canal; *Mn.* mandibles; *Mx.* maxillae.

fin ray of the host that it was not possible to remove the organ without breaking it at the point where the dotted ring appears in Fig. 3.

The *neck* is elongated and slender, showing slight segmentation anteriorly. The posterior portion, where it joins the trunk, is enlarged in a manner suggestive of *Medesicaste*.

The *trunk* is elongated and pyriform. Both it and the head are now of a deep brown colour. The genital region at the posterior end appears sharply marked off. The intestine in the trunk is characterised by the presence of

innumerable small saccate caeca, which also occur in *Lernaeocera cyprinacea*. In *Peniculus* there appears to be a single row of caeca along the dorsal wall of the intestine seemingly turned to one side in mounting (Fig. 2). One pair of small abdominal appendages is present.

The *appendages* (Fig. 3) are simple and reduced. The *antennules* are uniramous, 3-jointed, non-setigerous and contained in a niche of the carapace. The *antennae* are uniramous and 3-jointed, with spiny protuberances, one on the inner side of the proximal joint, a long one on the outer and two smaller on the inner side of the middle joint, and three setae at the tip of the terminal joint. The *mandibles* are of about the same width throughout their length, slightly curved at the apex, and serrate on one margin with about 8 teeth of equal size. The *maxillae* are stout with an expanded rounded apex bearing a large exopodite with an outer margin setigerous, and a small endopodite. Two pairs of *thoracic appendages* are present at the anterior end of the neck immediately posterior to the head, and two more pairs widely separated from the others, and situated on the neck at its swollen base (Fig. 2).

The entire length of the *alimentary canal* is visible (Figs. 2 and 3). The mouth and anus are distinctly defined, the former appearing triangular surrounded by a striated border.

The *reproductive system* (Fig. 2) is on much the same plan as in other parasitic Copepoda. The spermathecae are large, and the vulvae, instead of appearing close together near the middle line, are large crescentic slits near their apices. The ovaries are large and full of eggs. The oviducts are lengthy and convoluted, and enter the spermathecae near the middle line at a point opposite to that at which the egg-strings emerge.

7. CHONDRACANTHUS NODOSUS (Müller).

Material. 8 ♀♀ from *Sebastes norvegicus* (St. 1), on the gill arches, and in the angles formed by their junction with the gill covers.

This seems a variable species, and though the host, situation and appendages are normal, the lateral expansions of the trunk are more than usually well developed. The head of the animal is embedded in a tumour which is not in this case a hypertrophied gill filament, as it is in *Medesicaste*, but a hypertrophy of the skin of the gill arch.

In 1922 (*Parasitology*, XIV. 214) Leigh-Sharpe first drew attention to the presence of conjunctive tubes in *Medesicaste* Krøyer. In all of the *C. nodosus* ♀♀ there is a similar structure. Sometimes there is even a tube-like prolongation depending from a hypertrophy without any accompanying female: this is apparently formed by the embedded and concealed male which caused the hypertrophy.

Figure 4 A shows two specimens of *C. nodosus* ♀, *N* 1 (dorsal aspect) and *N* 2 (ventral aspect) whose heads are buried in a tumour *T*, the upper boundary of which is indicated in dotted outline, concealed by the flap *F*, of which it forms part, the whole being a hypertrophy in the angle of a gill-arch, and cut

from the host at and around the blackened portion which represents cartilage. Three conjunctive tubes are present: $C1$, which is probably abortive; $C2$, from the corner of the flap, and which has already or might subsequently have linked up with $N2$, and $C3$ which has already or might subsequently have linked up with $N1$, and which is extremely long, continued under the flap (where it is indicated by dotted lines), and arises right from the gill-arch. As the egg-sacs are full of ova it is presumed that the conjunctive tubes have linked up and are now disintegrating.

Fig. 4 B1 and B2 shows two positions of another specimen. Here the head is embedded in a tumour at the end of the row of gill-filaments in the angle of a gill-arch, cut from the host at the place where the blackened portion represents cartilage. There is, arising from the gill-arch, one accompanying conjunctive tube C which bifurcates, and which was actually attached to the

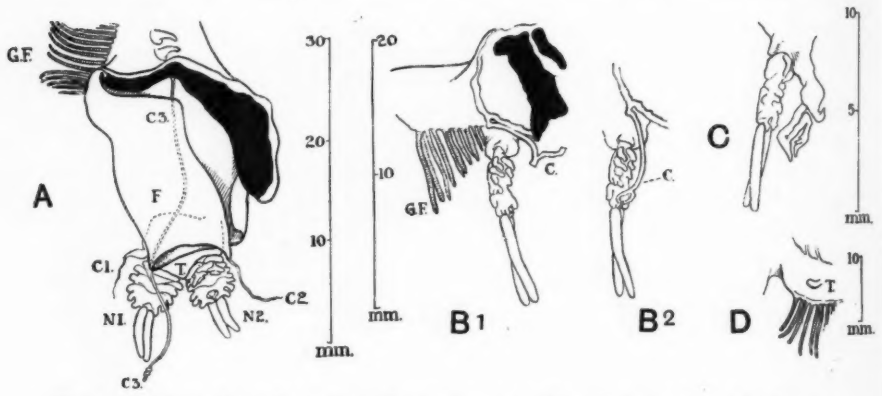

Fig. 4. *Chondracanthus nodosus*; for explanation see text. Two specimens ♀ $N1$ (dorsal aspect) and $N2$ (ventral aspect) embedded in a tumour T. concealed by a flap F. of which it forms part; C, $C1$, $C2$, $C3$, conjunctive tubes; $G.F.$ gill filaments.

vulvae of the female as in $B2$, one bifurcation to each vulva respectively. When detached one of the bifurcations broke off short and remained attached to the female as in $B1$.

Fig. 4 C shows an anomalous condition in which the female is embedded in one tumour while the conjunctive tube, which is very unequally bifurcated, springs from another at its side.

The important conclusion is that every one of these eight specimens (four of which are here figured) is accompanied by one or more attempts at a conjunctive tube, typically as in A.

Besides these tubes there are a number of incipient swellings situated actually on the gill-arch as in Fig. 4 D. These small tumours, when shaved off with a razor and mounted on a slide in Farrant's medium, are seen under the microscope to contain the remains of what appear to be male animals (Fig. 5 A).

As would be expected they do not closely resemble the normal epigynic males of *C. nodosus*, since they are entirely embedded and probably very degenerate. On tracing the conjunctive tubes into the tumour, they are found to arise from similar encapsulated structures (Fig. 5 B).

Although crudely preserved, sufficiently satisfactory transverse sections were obtained of some specimens. Fig. 6 B illustrates such sections of the tube below its bifurcation and shows that the structure consists of a cord of fibrous tissue which is practically spongiform through the great number of lacunae. Most of these lacunae are very small and retiform, but some are fairly large and follow an uninterrupted course for some little distance; many are filled with coagulated or colloid material. Under a moderate magnification (Fig. 6 A) the sections show certain very darkly-staining cells occurring in scattered groups usually in association with lacunae. Only one specimen was sufficiently

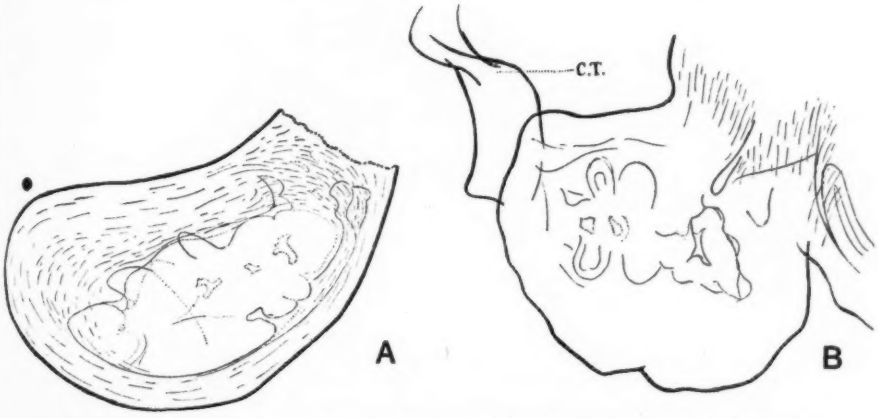

Fig. 5. *Chondracanthus nodosus.* Hypertrophies containing the remains of embedded male animals, mounted in Farrant's medium, and drawn under a microscope with camera lucida; *A* being the enlargement of Fig. 4 D. *C.T.* conjunctive tube.

well preserved for definite conclusions to be drawn from examination under $\frac{1}{12}$ inch objective. The tissue was found for the most part to consist of bundles of very elongated cells (*m*) which are arranged longitudinally in the central region (Fig. 6 B), and in a more circular manner at the periphery. These cells have elongate nuclei which are somewhat spider-like in cross-section; in longitudinal section they are seen to be composed of fibrillae, and altogether they strongly recall the plain muscle fibres of vertebrates. The fibre cells are mingled with much fibrous connective tissue (*f*) which unites them into bundles. Here and there, either mingled with the fibrous bundles, or applied to them, groups of deeply-staining glandular cells (*g*) are to be found; each mass contains several nuclei, but divisions between the cells could not be detected. Widely scattered between the bundles, or adhering to their

surfaces, occur certain small cells (*s*) consisting almost entirely of a deeply-staining nucleus with the scanty cytoplasm arranged in two or three projections so that the cell appears roughly triangular; in one favourably-placed cell the three projections are very regular, stiff and pointed, and slightly curved, suggesting the possibility that these cells are spermatozoa of a crustacean type.

The name originally applied to these cord-like structures in *Medesicaste* is here retained for the sake of continuity, but it must be emphasised that, here,

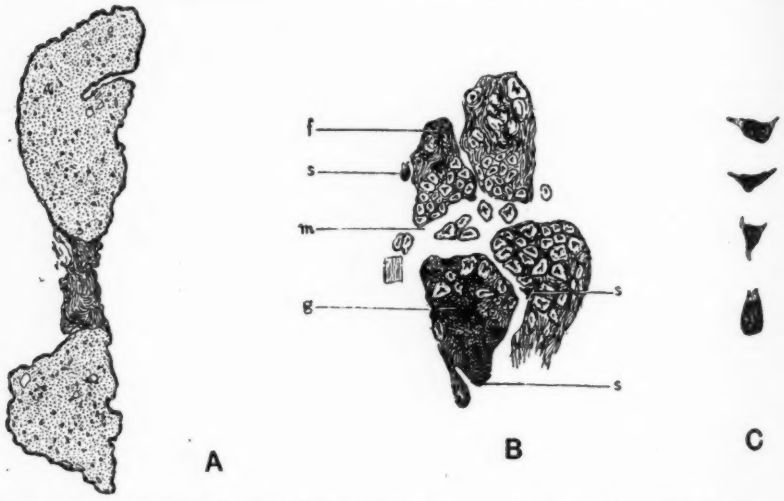

Fig. 6. *Chondracanthus nodosus*. A. Transverse section across *c* in Fig. 4 B at the point of bifurcation viewed under $\frac{1}{6}$ inch objective. B. A portion of the central region, showing *f.* fibrous tissue; *g.* glandular cells; *m.* elongated fibrous cells probably plain muscle; *s.* isolated ? spermatozoa. Viewed under $\frac{1}{12}$ in. oil immersion objective. C. Four examples of the possible spermatozoa, more highly magnified.

A and B are drawn with the aid of the camera lucida.

at any rate, they are functionally rather than anatomically tubes, and that they consist of spongiform fibrous tissue, including elongated cells which are perhaps muscular, glandular cells, and, in the interstices, possible spermatozoa.

8. CLAVELLA DUBIA T. and A. Scott.

Material. (*a*) 25 ♀♀ from the gill-filaments of *Gadus aeglefinus* (St. 1). (*b*) 2 ♀♀, one bearing 4 ♂♂ (one on the neck, one on the abdomen, one on each vulva) from the gill-filaments of *Gadus aeglefinus* (St. 3) (Fig. 7).

The determination of this species presents no difficulty, as the peculiar mandible, bipartite first maxillae, and extremely short second maxillae are unmistakeable. The genital process is robust and the *bulla is clavate.*

We do not think the males have been previously found.

Male. The male (Fig. 8) differs from that of *Clavella sciatherica* Leigh-Sharpe (*Parasitology*, XI. 123) and other species of *Clavella* we have seen, in having the front of the rounded dorsum flattened; this occurs in all four specimens and seems therefore not to be due to accident. The dorsum is expanded and well developed posteriorly. Fig. 8 was drawn from the best specimen, and in this the antenna is displaced so as to appear abnormally long, the other specimens show that it is no longer than in *C. sciatherica*; the mouth cone is not so elongated as in this species, but otherwise the appendages are similar. The second maxillae, previously wrongly called maxillipedes (*Parasitology*, XI. 266), are shorter and stouter. A specific feature is the very pronounced rostrum. The greatest length of the preserved male is about 0·4 mm.

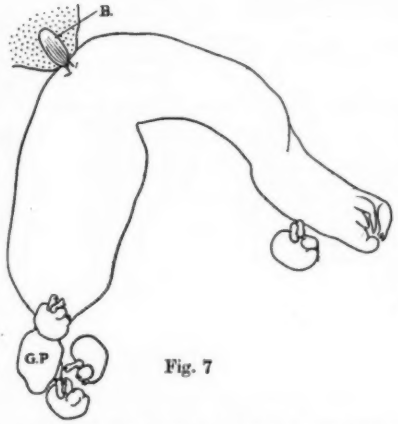

Fig. 7

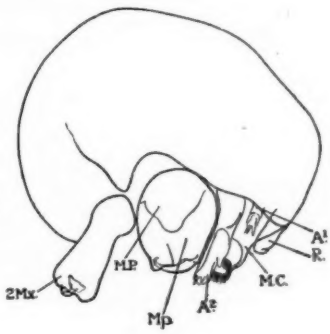

Fig. 8

Fig. 7. *Clavella dubia*, female bearing four males. *B.* bulla; *G.P.* genital process.

Fig. 8. *Clavella dubia*, male. *R.* rostrum; *A*1. antennule; *A*2. antenna; *M.C.* mouth cone; *Mp.* maxillipede; *M.P.* mediative process; 2*Mx.* second maxilla.

Female. One mature female specimen bearing two full-sized egg-sacs containing healthy ova was completely filled with numbers of curious metazoan parasites which are perhaps cercariae at a very early stage, since each possesses a multinuclear tail. A characteristic feature of these hyper-parasites is the inclusion of many small, fusiform, highly refractile bodies, possibly crystals.

9. CLAVELLA IADDA Leigh-Sharpe.

6 ♀♀ from the fins and tail, and 3 round the anus of the same *Gadus morrhua* (St. 1) (*Journal of the Marine Biol. Assn.* XII. 332).

Our thanks are due to Miss E. C. Humphreys for executing some of the figures.

(*MS. received for publication* 28. I. 1924.—Ed.)

ON A NEW GREGARINE, *GREGARINA SEGMENTATA* N. SP., AN INTESTINAL PARASITE OF *CIS BIDENTATUS* OLIVIER (COLEOPTERA).

By MARY VINCENT.

(*From the Molteno Institute for Research in Parasitology, University of Cambridge.*)

(With 9 Text-figures.)

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Material.

Cis bidentatus, which acts as host to this gregarine, is a small fungus-eating beetle. Almost all the beetles examined proved to be infected, but the parasites were never very numerous in each host. As a rule the infected beetles contained from four to eight of the sporont associations, and sometimes a few of the cephalont stages or cysts. The adult gregarines may be seen by transparency through the wall of the mesenteron: they lie either straight or else bent in the form of a U, but always with their long axis parallel to the sides of the gut. They are restricted to the posterior region of the midgut, and are never distributed along its whole length.

Two of the beetles showed in addition an infection of the malpighian tubules with a Schizogregarine of the genus *Ophyrocystis*.

Trophozoites.

The earliest stages of *Gregarina segmentata* which were observed were the young cephalonts (Figs. 1, 2 and 3). These are provided with a simple symmetrical epimerite somewhat swollen at its base. In its young stages the trophozoite conforms to the usual type of tricystid gregarine; that is to say, the body is divided into the three divisions, epimerite, protomerite, and deutomerite. As the animal increases in size, however, the deutomerite

becomes secondarily segmented, and the next stage seen was a cephalont in which the deutomerite was divided into three compartments by two septa at right angles to the axis of the body (Fig. 4).

The cephalonts were rare in my material and further intermediate stages were not seen, but it is probable that the secondary segmentation does not normally proceed far in this stage.

SPORONTS.

The sporonts associate in couples, the tail of the primite fitting into a depression in the protomerite of the satellite. The gregarines are long and slender, and the association bears a superficial resemblance to a small segmented worm (Figs. 5 and 6). It is usually easy to distinguish the protomerite and deutomerite in each member of the association because the septa which divide them are rather more clearly defined than those which separate the secondary segments.

When first examined in normal salt solution the gregarines are moderately active and move forward with a slow gliding motion.

The total length of the associations varies between 100 and 560 μ, with an average of 375 μ: the average width is 15 μ. The ratio of length of protomerite to total length is 1 : 3, and the ratio of width of protomerite to width of deutomerite is 1 : 1.

In each member of the association there is a single ellipsoidal nucleus visible as a transparent area in the living specimen. The position of the nucleus in the deutomerite is variable: usually it is situated at the anterior end, sometimes at the extreme posterior extremity, but very rarely in an intermediate position. In young specimens the nucleus contains a single spherical karyosome, but in more mature forms, owing to a process of budding, the karyosome becomes irregular or pear-shaped. Inside the nuclear membrane there is a ring of chromatin (Fig. 7).

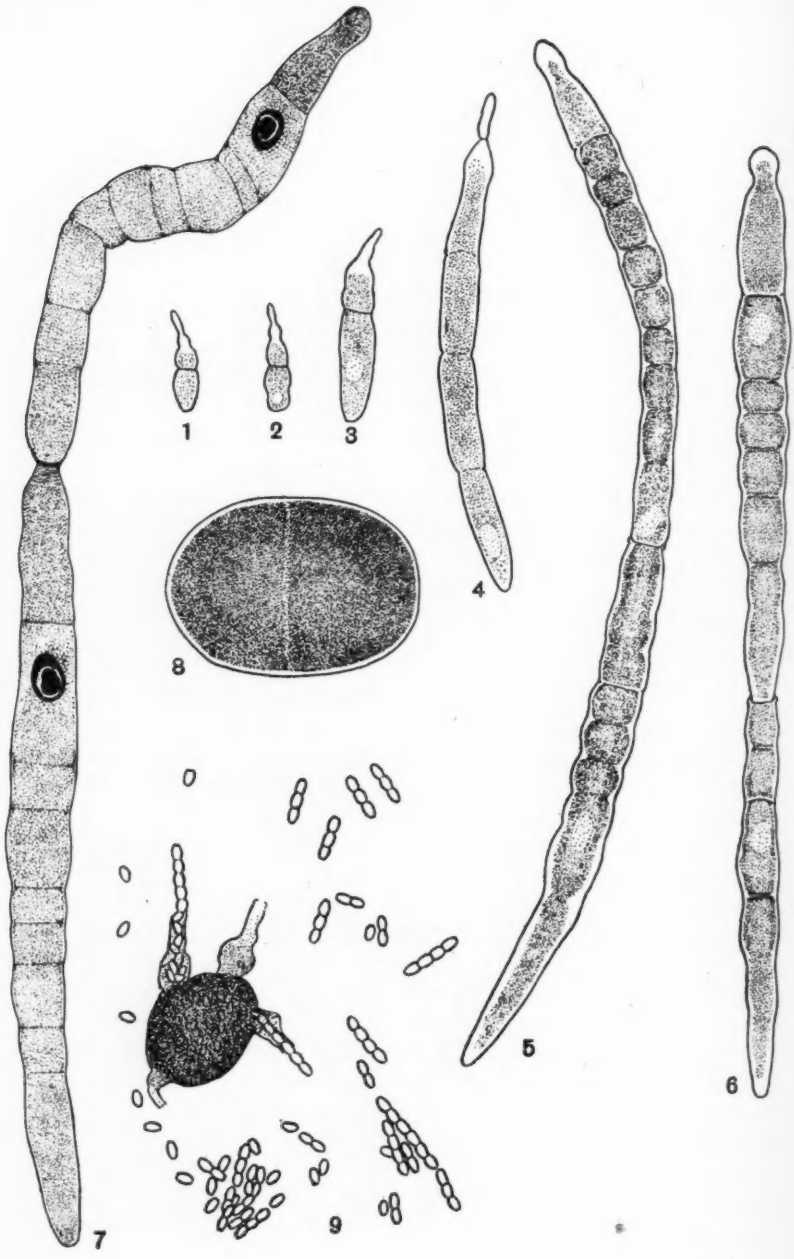
The endoplasm is filled with fairly coarse granules, and the animal has a greenish-yellow colour when viewed by transmitted light. The epicyte is thick and is ornamented with fine longitudinal striations.

The most interesting feature of the gregarine is the secondary segmentation. The number of segments is variable, and there is not the constant relation between the number of segments and the size of the gregarine which Léger (1906) found to exist in the somewhat similar form *Taeniocystis*.

In the primite the segmentation is confined to the deutomerite, but in the satellite the protomerite may also be segmented.

The septa are always horizontal, and they are accompanied by a constriction of the integument. Some of the septa are much more clearly defined than others, and those in the posterior region of the body are often difficult to distinguish. The septa are not uniformly distributed and consequently the segments vary in length.

It is difficult to determine to what depth the septa extend, and whether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they form a complete wall or are of the nature of a diaphragm. That they are not merely superficial structures is evident from the fact that they are clearly visible in sections of the gregarine, and in some cases at least, it seems probable that they constitute a complete partition. The septa stain strongly with haematoxylin and are conspicuous in toto mounts.

CYSTS AND SPORES.

The cysts collected from the intestine or from the excreta are ellipsoidal in shape, and have an average measurement of $80 \times 60\,\mu$. Cysts were incubated on a slide in a moist chamber, and although the majority of them disintegrated, a few completed their development.

The spores are barrel-shaped and measure $5 \times 3\,\mu$, and they are extruded from the cyst through spore-ducts (Fig. 9). In most of the ripened cysts only four ducts were developed, but in two of the cysts there were indications of six ducts.

SYSTEMATIC POSITION.

This gregarine may clearly be assigned to the genus *Gregarina*, Family Gregarinidae. The association of the sporonts in couples, the simple epimerite, the method of cyst dehiscence, and the barrel-shaped spores are all highly characteristic of this genus. The only peculiarity of the present species is the segmentation, which is undoubtedly a secondary character caused by environmental conditions, and it is unnecessary on this account to create a new genus

COMPARISON WITH OTHER SEGMENTED GREGARINES.

A similar segmented condition of the body is very rare amongst gregarines and is without parallel in the family Gregarinidae. A list of species which show a comparable structure is given below:

	Host	Habitat
Gregarina (?) *annulata* Greef	*Rhynchonerella fulgens* (Polychaete)	Gut
Taeniocystis mira Léger	*Ceratopogon solstitialis* (Diptera)	,,
Taeniocystis légeri Cognetti de Martiis	*Kynotus pitarellii* (Oligochaete)	Coelome
Metamera schubergi Lyndhurst Duke	*Glossosiphonia complanata* (Hirudinea)	Gut
Dendrorhynchus systeni Keilin	*Systenus scholtzi* and *S. adpropinquans* (Diptera)	,,
Gregarina segmentata n. sp.	*Cis bidentatus* (Coleoptera)	,,

LEGENDS TO TEXT-FIGURES.

Figs. 1–9. *Gregarina segmentata* n. sp.

Figs. 1, 2 and 3. Young cephalonts. ×450.
Fig. 4. Cephalont showing two secondary septa. ×450.
Figs. 5 and 6. Sporont associations. ×450.
Fig. 7. Sporont association from a smear fixed in Schaudinn and stained in iron-haematoxylin ×550.
Fig. 8. Young cyst. ×450.
Fig. 9. Ripe cyst discharging spores. ×450.

All the figures except Fig. 7 were drawn from living specimens

The form described by Greef (1885) from the intestine of the Polychaete *Rhynchonerella fulgens* has the body divided into a number of segments, but it is so briefly described and incompletely figured as to be of no use for purposes of comparison. It certainly does not belong to the genus *Gregarina*, but, lacking sufficient data, it cannot be assigned to its correct position.

Taeniocystis mira, discovered by Léger (1906) in the intestine of the dipterous larva *Ceratopogon solstitialis*, is the classical example of a metameric gregarine and has been described by him in detail. He found that the membranes of the partitions in this species are formed by the chemical modification of excretory granules which collect in a ring at the spot where the septum is to arise. In *Gregarina segmentata* the septa do not appear to be formed in this manner, but by an ingrowth of the ectoplasm.

T. légeri, described by Cognetti de Martiis (1911), from the coelome of the Oligochaete *Kynotus pitarellii*, has at least a superficial resemblance to *T. mira*, but only the trophozoite stages have been described, and those from six fixed specimens. Kamm (1922) does not place it with the type for the genus, and apparently doubts its position among the gregarines: "The protomerite is divided into three segments, which is unique. The parasite is coelomic rather than intestinal, as are all other polycystid gregarines. The epimerite is unknown. For these reasons it is not placed with the type for the genus." With regard to her first objection, namely, the segmentation of the protomerite, such a condition occurs frequently in the satellite of the associations of *G. segmentata*. It is conceivable that the forms observed by Cognetti de Martiis were degenerating specimens of a monocystid gregarine, which would explain their coelomic position, cf. *Rhynchocystis pilosa*, mentioned below.

In *Metamera schubergi*, discovered by Lyndhurst Duke (1910) in the gut of the leeches *Glossosiphonia complanata* and *Hemiclepsis marginata*, the segmentation is confined to the posterior region of the deutomerite, and is not always present. The writer discusses the possibility of the appearance being due to plasmolysis, but he considers that the evidence does not support this view. The septa were not seen in any sections of the gregarines, and nothing is stated as to their structural formation.

Hesse (1909) has noted a curious segmentation of the body in degenerating forms of the monocystid gregarine *Rhynchocystis pilosa*, parasitic in the vesicula seminalis of earthworms. Such gregarines show a marked resemblance to *Taeniocystis*, but the septa arise in a different manner, being formed by annular thickenings of the sarcocyte which penetrate into the endoplasm. He considers that the segmentation in this case is due to mechanical conditions, and not to trophic phenomena, since it is only present in degenerating parasites in which nutrition has ceased. The possibility that the septa of other species are also of pathological origin must not be overlooked, but in *Gregarina segmentata* they are certainly not abnormal structures because they are invariably present, and the parasites show no signs of degeneration.

Finally, mention must be made of *Dendrorhynchus systeni* Keilin (1920)[1] from the gut of the dipterous larva *Systenus*. In this gregarine the body is surrounded by transverse anastomosing fibrils which the writer compares to the myocyte fibrils of *G. munieri* described by Schneider, and he states: "In some specimens of *Dendrorhynchus* it was rather difficult to ascertain that the transverse fibrils were only superficial and did not penetrate deeper in the endoplasm and form a series of septa similar to those of *T. mira* described by Léger (1906)." Dr Keilin has kindly allowed me to examine his preparations of *Dendrorhynchus*. The transverse fibrils are clearly visible in sections of the gregarine and are not unlike the septa of *G. segmentata* except that they are much closer together and have anastomosing branches.

It is rather difficult to account for the convergence in structure exhibited by these different gregarines. Léger (1906) in discussing the only two segmented forms then known, *G. annulata* and *T. mira*, instances these as examples of the curious superficial resemblance which exists between the parasites of Annelids and those of dipterous larvae; a resemblance which he considers may be due to a similarity in the biomechanical conditions of their environment. It is interesting to note that of the segmented gregarines since described *G. segmentata* is the only species whose host does not belong to one or other of these groups.

In *T. mira* Léger considers that the primary cause of the segmentation is mechanical, and that it may be due to movements of the gregarine itself, to the peristalsis of the intestine, or to movements of the host as a whole: the septa, however, have secondarily a trophic origin since they are formed by the modification of excretory granules.

"On peut supposer que ces mouvements exercent peu à peu une sorte de dislocation, de tronçonnement dans l'ensemble des éléments endoplasmiques déterminant par places, à des intervalles réguliers des zones de cytoplasma d'abord libres d'enclaves au niveau desquelles s'accumulent ensuite les grains d'excrétion de taille très exigue et de densité différente de celle des corpuscules de réserve. Ainsi, des phénomènes d'ordre trophique s'ajouteraient aux premiers pour déterminer le cloisonnement de l'organisme."

It is questionable whether the segmentation of the other septate forms can be explained in a like manner. It is certainly difficult to imagine that environmental conditions are similar in each case, especially for *G. segmentata*, which is parasitic in the gut of a beetle. Moreover, the septa in the various species are apparently formed in different manners (from endoplasm in *T. mira*, and from ectoplasm in *D. systeni* and *G. segmentata*) and are not therefore truly homologous structures, even though the primary cause of the segmentation may possibly be similar.

[1] In Prof. Léger's opinion this gregarine should be ascribed to the family Actinocephalidae, and not to the family Dactylophoridae, where it was provisionally placed by Dr Keilin.

DIAGNOSIS OF *GREGARINA SEGMENTATA* n. sp.

Characters of genus *Gregarina*.

Sporonts elongated, and divided into a number of segments by transverse septa. Average length of associations 375 μ, and width 15 μ. Ratio of length of protomerite to total length 1 : 3, and ratio of width of protomerite to width of deutomerite 1 : 1.

Cysts ellipsoidal, 80 × 60 μ, dehiscence by sporoducts.

Spores barrel-shaped, 5 × 3 μ.

Habitat: midgut of *Cis bidentatus* Olivier (Coleoptera).

Locality: Cambridge, England.

ACKNOWLEDGEMENTS.

My thanks are due to Dr D. Keilin for his helpful advice and criticism, and also to Dr H. Scott, who kindly identified the host beetle.

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THE TAPEWORMS OF THE RANGOON PIGEON.

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(With Plate XIII and 5 Text-figs.)

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The pigeons of India are notoriously heavily infected with tapeworms and a cursory examination showed those of Rangoon to be no exception. A systematic investigation, the results of which are given in the accompanying table, was made of 20 pigeons taken from various parts of the town. Those from some localities were comparatively free from infection, others heavily parasitised. On the whole, those kept in cages and fed on corn had the greatest percentage of worms. The effect upon the health was very pronounced. Extreme emaciation and weakness were the usual accompaniment to a heavy infection. The worms were mostly situated in the duodenum, *Hymenolepis* nearer the stomach, *Cotugnia* and *Raillietina* further away, but this arrangement was liable to be altered. In one case, a bird on the point of death, the worms had moved nearly to the anus, some remaining free, others attached, indicating the probability under normal conditions of the cestode moving from place to place.

Pigeon	*Hymenolepis*	*Houttuynia*	*C.* var. *tenuis*	*C.* var. *nervosa*
115	Numerous	Numerous	—	—
122	—	—	—	—
123	—	—	—	—
124	Numerous	—	—	3
125	Present	—	—	—
126	Present	—	3	—
127	—	—	2	—
128	—	—	—	—
129	Numerous	4	3	—
130	Numerous	—	1	—
133	—	—	—	—
134	—	—	—	7
135	Present	—	—	—
138	1	—	—	—
139	—	—	—	—
140	—	—	—	—
141	—	3	—	—
142	—	—	—	—
143	—	—	—	—
144	—	2	—	—

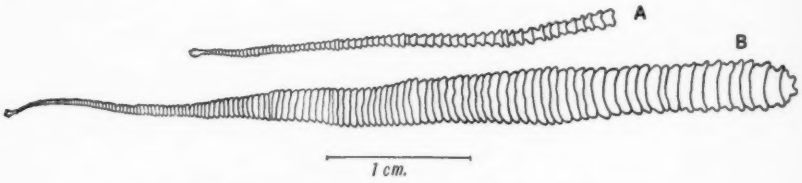
Percentage of infections:

Uninfected	*Hymenolepis*	*Houttuynia*	*C.* var. *tenuis*	*C.* var. *nervosa*
40	40	20	20	10

Co-types of *Houttuynia torquata*, *Cotugnia cuneata nervosa* and *Hymenolepis rugosa birmanica* sent to the Molteno Institute for Research in Parasitology Cambridge, the British Museum, and the Bureau of Animal Industry, Washington, and of *C. cuneata tenuis* to the first museum.

Cotugnia cuneata n. sp. var. *tenuis*.

Length 30[1], maximum breadth 1·00. Scolex 0·26 diam., rostellum armed with a double circle (0·12 diam.) of approximately 400 hooks, 0·014 and 0·018 long, the long and short hooks alternating. Posterior to rostellum a circular cushion as in other species. Suckers unarmed. Body small, proglottides posteriorly longer than broad. Musculature weak, consisting of two layers of longitudinal muscles, each between a weak transverse one. Inner longitudinal layer on each side consisting of approximately 15 bundles well separated from one another, normally each formed of 20 fibres but fewer in those external to the excretory vessels. Outer layer composed of small bundles more tightly packed together.



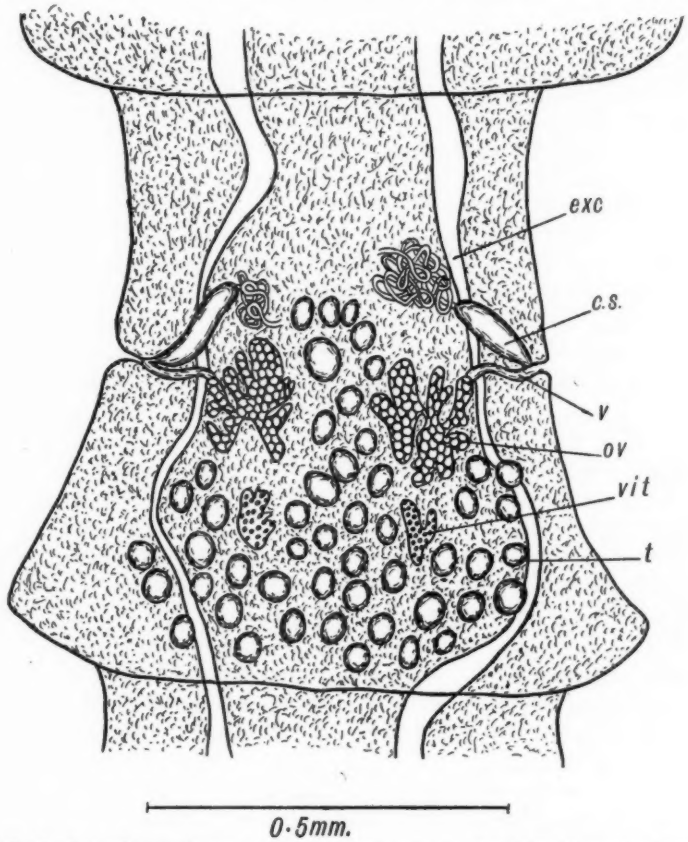
Text-fig. 1. *Cotugnia cuneata*. *A*, var. *tenuis*; *B*, var. *nervosa*.

Genital pores (Text-fig. 2) usually in the centre of the proglottis margin but vary slightly in position, being sometimes more anterior, sometimes posterior. Cirrus-sac large, extending immediately internal to or as far as ventral excretory vessel. Testes along posterior border of segment, in a quadruple row extending laterally to the excretory vessels and in the centre of the proglottis being augmented sufficiently to stretch anteriorly to the level of the genital pore. Vaginal pore posterior to cirrus-sac. Each ovary a much lobed crescent, the concavity directed posteriorly. Ovaries mostly close to excretory vessel separated by a clear space, but often close together and almost touching, the exact state being dependent upon the degree of contraction of the proglottis. The portion of the segment anterior to genital pore devoid of genital organs. Eggs singly in thick walled capsules extending laterally beyond and surrounding the excretory vessels. Wall of capsule unusually thick and deeply staining.

At the posterior end of one specimen, the cirrus-sac of one side showed considerable variation in position, moving from the centre of the proglottis margin to the extreme anterior corner, finally vanishing together with the

[1] All measurements in mm.

genital organs of that side. The other side was normal in all respects except that upon the disappearance of the ovary of the opposite side, the genital organs increased in size and moved to the centre of the proglottis, the general appearance being that of a typical *Raillietina* segment.



Text-fig. 2. *Cotugnia cuneata* var. *tenuis*. *c.s.*, cirrus-sac; *exc.*, longitudinal excretory vessel; *ov.*, ovary; *t.*, testis; *v.*, vagina; *vit.*, vitelline gland.

C. cuneata var. *nervosa*.

This variety is similar to the former in most respects, differing in the larger size of the strobilus, the transversely elongated proglottides and the great development of the musculature. Length 60, greatest breadth 3. Scolex extraordinarily tightly fixed to intestine of host. All proglottides considerably broader than long and very thick. Musculature consisting of three layers of longitudinal muscles, each bounded internally by transverse ones. Inner layer with a double, often treble, series of bundles each with 20 large fibres, the

bundles being intermingled with one another and tightly packed. Bundles of middle layer smaller, composed of fewer fibres and not so crowded; those of outer still smaller, merging into a few scattered fibres under the subcuticula. Transverse musculature weak.

Referring to a key previously given (Meggitt, 1920, p. 306) it will be seen that the species most closely allied is *C. fuhrmanni* Baczynska (1914, p. 202). From this the form described differs in the number (Meggitt, p. 400; Baczynska, p. 200), size (0·014 and 0·018—Baczynska, 0·015) and arrangement (alternating sizes—one size) of the hooks, the possession of unarmed suckers and of a vagina opening posteriorly to the cirrus-sac. It is therefore necessary to create for it a new species for which I propose the name *C. cuneata*. In spite of the striking contrast in appearance, the two forms of this species agree in number and size of hooks and in the arrangement of the genital organs, the differences being confined to size and musculature and being therefore insufficient to justify the creation of separate species. With regard to their occurrence, while var. *tenuis* has rarely been found except singly (maximum number 3) and usually mixed with numerous *Hymenolepis rugosa*, var. *nervosa* has always occurred alone and often in large numbers.

Musculature of the genus *Cotugnia*.

The musculature of the genus *Cotugnia* (Plate XIII) shows surprising differences between species, varying from extreme weakness to great strength. *C. brotogerys* Meggitt, 1915 (Fig. 4), has the feeblest. Here the musculature consists of three layers of longitudinal, each limited internally by transverse muscles. There are few bundles, each longitudinal stratum consisting of single fibres with a tendency towards aggregation but often scattered. In the scolex they are relatively large. Posteriorly the transverse muscles increase in size but the longitudinal diminish. *C. cuneata* var. *tenuis* (Fig. 6) shows a further stage. The inner longitudinal muscles are small with few fibres while external to them are smaller scattered bundles and fibres extending into the cuticula. Transverse muscles limit the inner longitudinal muscle layer. In *C. digonopora* (Pasquale, 1890) (Fig. 3) are two bands of longitudinal and transverse muscles, the inner layer of the former consisting of relatively large bundles, each of 20 fibres, and each separated from its neighbours by well-developed dorso-ventral muscles. Outer layer of transverse muscles double and very strong, that of longitudinal muscles with bundles varying from 10 to single fibres according to the distance from the cuticle: these decrease in size posteriorly. The dorso-ventral muscles are well developed throughout the strobilus. In *C. fuhrmanni* Baczynska, 1914, are three longitudinal muscle layers—the inner with well-developed bundles of 50 fibres, those of the central with 11, the outer consisting of scattered isolated fibres—each limited internally by transverse ones. The musculature of *C. fastigata* Meggitt, 1920 (Fig. 5) is similar. In *C. cuneata* var. *nervosa* (Fig. 1) it is of greater complexity although of smaller size: in it are three longitudinal and three transverse bands as before, but the former

consist almost entirely of bundles, there being practically no scattered fibres. The inner layer is single or treble according to the state of contraction of the strobilus. The muscles here occupy all the thickness of the proglottis except a narrow strip, one-fifth of the dorso-ventral diameter, a remarkable contrast to *C. brotogerys*. The greatest development is in *C. crassa* Fuhrmann, 1908, with 4–5 zones of longitudinal, separated by three of transverse muscles, the inner longitudinal layer being single or treble according to the degree of anastomosis at the point taken, or in *C. polycantha* Fuhrmann, 1908 (Fig. 2) with a fourth transverse band. A variation is shown by *C. collini* Fuhrmann, 1908, which has the transverse muscles extraordinarily well developed, the fibres pervading the entire cortical parenchyma and penetrating between the longitudinal bundles. The latter are apparently feebly developed but could not be investigated thoroughly by reason of the poor fixation of the specimens. It may be seen from the above brief summary that the musculature of the species of this genus may be used for identification. When the variations in one individual have been correlated with the position in the strobilus this character will be of use in naming small fragments devoid of scolex or distinguishing genital organs.

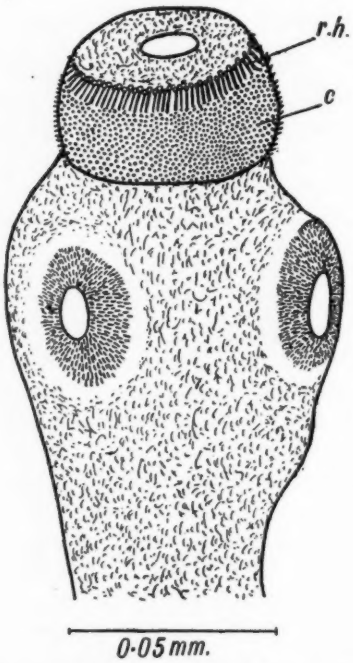
Houttuynia torquata n. sp.

Length 230, greatest breadth 2·5. Scolex 0·09–0·095 diam. (Text-fig. 3). Rostellar hooks 150, 0·007 and 0·0075 long, the two sizes alternating, the anterior extremity of the smaller hooks being slightly behind that of the layer: circle of hooks 0·042–0·045 diam. Posterior border of rostellum not extending past centre of suckers. Posterior to and surrounding the rostellum a barrel-shaped collar, thickly studded with minute spines which are easily lost, similar to that figured by Dujardin (1845, Pl. IX, fig. L 1) for *H. frontina*. Suckers armed with thorn-shaped hooks, 0·008 long, arranged in approximately five rows, absent from posterior border of sucker. Terminal proglottides rather longer than broad. Musculature weak, consisting of an inner double layer of transverse muscles surrounded externally, on each side of the segment, by 13–18 longitudinal muscle bundles, each consisting of 3–5 fibres. External to these a second scattered layer of single fibres extending to the sub-cuticula. Scattered dorso-ventral muscles present. Excretory system with the usual four longitudinal vessels: in immature segments a fifth, poral or central may appear.

Genital pores unilateral (Text-fig. 4) in centre of proglottis margin or slightly posterior to it. Genital cloaca small. Testes 8–10, two poral, one posterior to female organs, the remainder aporal. Cirrus-sac small, reaching longitudinal nerve and containing a small vesicula seminalis. Cirrus armed. Vas deferens coiled more than usual. Receptaculum seminis absent. Ovary bilobed, each lobe much sub-divided, extending two-thirds of the distance between the ventral longitudinal excretory vessels. Uterus quickly breaks down and eggs pass into parenchyma, later to become embedded in groups of 3–5 in

egg capsules. Egg-capsules 30–40 in each proglottis, extending just externally to longitudinal excretory vessels.

Among the species of the genus *Davainea* (Blanchard, 1891, nec Fuhrmann, 1920) only two (*Taenia frontina* Dujardin, 1845, and *T. struthionis* Parona, 1885) are recorded with a collar of spines posterior to the rostellum. From the latter the present species is separated by the possession of acetabular hooks and a large cirrus-sac, from the former by the smaller number (150 compared with 250) and size (0·007 compared with 0·008, 0·01 and 0·014 of authors)

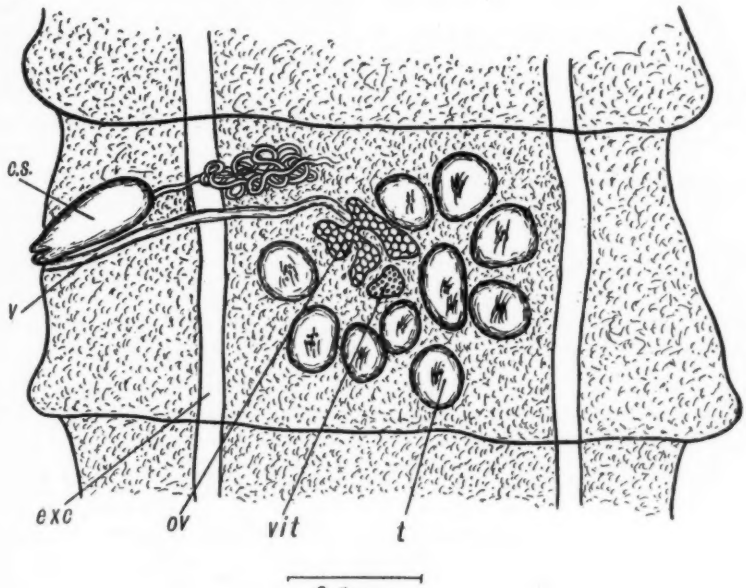


Text-fig. 3. *Houttuynia torquata.* c., spined collar; r.h., rostellar hooks.

of the rostellar hooks and the smaller number of the testes (8–10 compared with 50). These differences, together with the absence of the first two forms from the *Columbiformes*, appear to justify, for the present specimens, the creation of a new species for which I propose the name *Houttuynia torquata*.

The genus *Houttuynia* was originally created by Fuhrmann (1920) with *T. struthionis* as the type species and described as "Davaineinae: De grande taille. Scolex armé d'un gros rostellum avec deux rangées de grands crochets et plusieurs séries d'épines. Venteuses inermés (?). Pores sexuels unilatéraux. Poche du cirre de forme très allongée. Testicules très nombreux. Glandes sexuelles femelles rapprochées du pore sexuel. Capsules uterines avec plusieurs

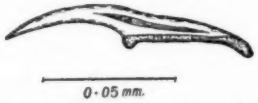
oeufs." *T. frontina* was provisionally included. In view of the restricted occurrence of the spined collar in the old genus *Davainea*, to place forms possessing it in different genera seems unnatural. I would suggest that the genus be re-defined by the omission of "Poche du cirre...oeufs" to allow of the inclusion of *D. beddardi* Meggitt, 1921, *D. frontina* (Dujardin, 1845), *D. linstowi* Meggitt, 1921, and *D. torquata*, n. sp.



Text-fig. 4. *Houttuynia torquata*, lettering as in Text-fig. 2.

Hymenolepis rugosa Clerc, 1906, var. *birmanica* nov. var.

Length 70, breadth 0·6. Scolex 0·21 diam., with four unarmed suckers and a rostellum. Rostellar hooks (Text-fig. 5) 8, 0·088–0·095 long, differing from those of *H. rugosa* Clerc (1906, 433) in size and in having the proximal end slightly bent. Rostellar sheath extending posteriorly past suckers.



Text-fig. 5. *Hymenolepis rugosa* var. *birmanica*, rostellar hook.

Musculature consisting of an outer continuous and well-developed longitudinal layer, the bundles composing which contain 7–9 fibres and are not well separated from each other: internal to it a slender sheath of transverse muscles

followed by eight longitudinal muscle strands each of the same size as the bundles of the first layer and arranged two between the excretory vessels and two external to them on each surface. A second slender sheath of transverse muscles separates cortical and medullary parenchyma.

Genital pore in extreme anterior angle of the proglottis margin. Cirrus-sac and vagina open into a small genital cloaca. The former extends beyond the aporal excretory vessel, in certain cases almost to the aporal proglottis margin and is surrounded externally by a very definite pavement epithelium. Cirrus armed, its long straight chitinous lumen forming the "anguille chitineuse" of Clerc. Internal and external vesiculae seminales small, the former occupying half the cirrus-sac, the latter extending from the aporal excretory vessel one-third along the cirrus. Testes dorsal, usually in a straight line across the proglottis but the arrangement of the aporal two is liable to great variation, one testis being anterior, ventral or posterior to the other. Ovary slightly lobed, touching when fully developed the excretory vessels of each side. Receptaculum seminis small, extending from poral excretory vessel to centre of proglottis.

The species just described obviously closely resembles *H. rugosa* Clerc, 1906, but differs from it in the size (0·088–0·095 compared with ·00–·102) and shape of the rostellar hooks. The hooks of the specimens seen varied 7 μ in length: this is also the difference between their greatest length and that given by Clerc. As the other anatomical details agree, it is best to consider this form a variety of *H. rugosa* for which I propose the name *birmanica*.

H. sphenocephala (Rudolphi, 1810).

This species has previously been recorded from Rangoon pigeons (Meggitt, 1920, p. 306) but no examples were found this time in the pigeons dissected.

Tapeworms of Columbidae.

Names of hosts as given in Sharp (1899):

Columba guinea L.

Raillietina senaariensis (Weithofer, 1916, p. 313).

C. gymnophthalma Temm. and Knip.

Bertiella delafondi (Railliet, 1892). Fuhrmann, 1908, p. 130.

Hymenolepis armata Fuhrmann, 1906, p. 353.

C. leucocoronata Vig.

Raillietina ceylonica (Baczynska, 1914). Southwell, 1922, p. 137.

C. livia Bonn.

Bertiella delafondi (Railliet, 1892). Fuhrmann, 1902, p. 132.

Hymenolepis rugosa Clerc, 1906, p. 433.

Ligula sp. Stiles, 1896, p. 10.

Raillietina crassula (Rudolphi, 1819). Fuhrmann, 1909, p. 104. Meggitt, 1921, p. 12.

R. crassula (Clerc, 1906, p. 725). Blanchard, 1891, p. 436. Meggitt, 1921, p. 12. Stiles, 1896, p. 53.

C. livia domestica.

Bertiella delafondi (Railliet, 1892, p. 49). Fuhrmann, 1901, p. 759; 1902, p. 132. Johnston, 1918, p. 168. Stiles, 1896, p. 10. Wolffhügel, 1904, p. 45.

Bothriocephalus columbarum Bertkau. Stiles, 1896, p. 26.

Tapeworms of Columbidae (continued).

C. livia domestica (continued).

Hymenolepis sphenocephala (Rudolphi, 1810, p. 94). Fuhrmann, 1906, p. 448. Stiles, 1896, p. 53.

Raillietina crassula (Rudolphi, 1819). } As
R. crassula (Clerc, 1906). } before

Taenia cordifera Bosc. 1802, p. 306.

C. palumbus L.

Raillietina columbae (Fuhrmann, 1909, p. 104).

C. sp.

Cittotaenia columbae Skrjabin, 1915, p. 64.

Dipylidum columbae Fuhrmann, 1906, p. 81.

Raillietina anatina (Fuhrmann, 1908). Southwell, 1922, p. 136.

R. cryptacantha (Fuhrmann, 1909, p. 105).

R. spiralis (Baczynska, 1914, p. 198). Southwell, 1922, p. 136.

Ectopistes migratorius L.

Bertiella delafondi (Railliet, 1892). Fuhrmann, 1902, p. 132.

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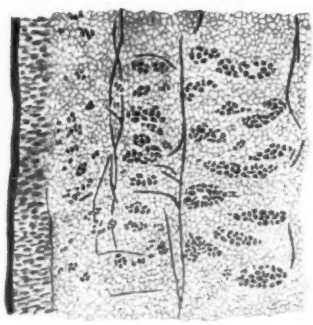
DESCRIPTION OF PLATE XIII.

All figures the same magnification except Fig. 2.

Fig. 1. *Cotugnia cuneata* var. *nervosa.*
Fig. 2. *C. polycantha* (after Fuhrmann, scale not given).
Fig. 3. *C. digonopora.*
Fig. 4. *C. brotogerys.*
Fig. 5. *C. fastigata.*
Fig. 6. *C. cuneata* var. *tenuis.*



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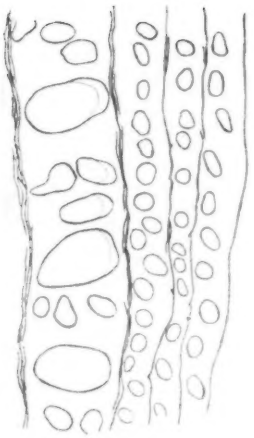

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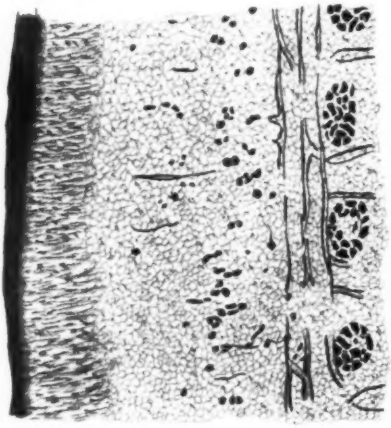

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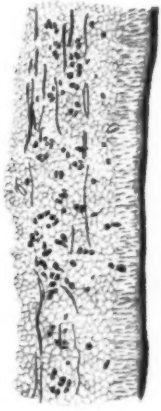

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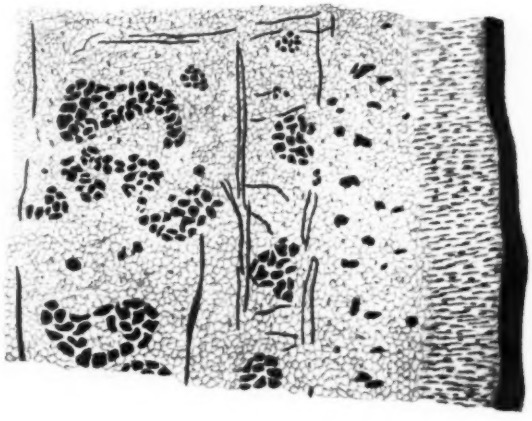

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DIE VERWANDTSCHAFTSBEZIEHUNGEN DER ZECKENGATTUNGEN[1].

VERSUCH EINES NATÜRLICHEN SYSTEMS AUF VERGLEICHEND-MORPHOLOGISCHER GRUNDLAGE.

Von ERICH JACOB.

(*Rostock.*)

Eine besonders geeignete Gruppe zur Untersuchung verwandtschaftlicher Beziehungen und zur Feststellung der Wege, die bei der Art- und Gattungsbildung eingeschlagen werden, scheinen gerade die Zecken zu sein, da sie alle unter verhältnismässig gleichartigen Bedingungen leben und in nur wenige Gattungen zerfallen, die aber zahlreiche Arten aufweisen.—Die Überlegungen, die zu dieser Arbeit führten, waren kurz folgende:

An Hand von offenbar nicht miteinander in Beziehung stehenden Merkmalen sollte das Verwandtschaftsverhältnis der einzelnen Gattungen ergründet werden, und zwar so, dass die mit Hilfe eines Merkmales gefundenen Beziehungen von einem zweiten, dritten usw. Merkmale, das von dem ersten in keiner Weise beeinflusst erscheint, verneint oder bestätigt werden. Als Merkmale sind solche herausgegriffen worden, die bis jetzt ganz ungenügend oder überhaupt noch nicht einer genaueren Untersuchung unterzogen worden waren. Der erste Teil der Arbeit handelt also von jenen Merkmalen, der zweite von den Folgerungen, die als Ergebnis der Untersuchungen für die einzelnen Gattungen gezogen werden müssen, und der dritte Teil endlich davon, wie sich bis jetzt die Forscher auf Grund ihrer Veröffentlichungen den stammesgeschichtlichen Aufbau des Zeckenreiches dachten, und wie weit diese Ansichten mit meinen Ergebnissen übereinstimmen. In einem Anhange wird eine neue, interessante Farbvarietät von *Dermacentor rhinocerinus* Denny beschrieben, die mir bei der Durchsicht des umfangreichen Materials des Museums für Naturkunde, Berlin, in die Hände fiel.

Die zur Untersuchung herangezogenen Merkmale sind zuerst die Furchenbildungen auf dem Scutum, Alloscutum und der Ventralseite der Zecken. An diesen Furchen inseriert die Dorsoventralmuskulatur, die stets zwischen den einzelnen Darmverzweigungen zu liegen kommt. Bereits 1923 wies P. Schulze darauf hin, dass *diese Darmdivertikel die Folge von im Embryo auftretenden Faltenbildungen der Darmblätter des Coeloms sind und sich also in letzter Linie jene Furchen und die von diesen abhängigen Zeichnungselemente vieler Zecken*

[1] Auszug aus der Rostocker Inaugural-Dissertation.

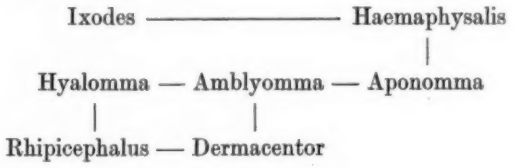
auf embryonale Verhältnisse zurückführen lassen, mithin organisatorische, nicht adaptive Merkmale sind. Es stellte sich nun heraus, dass alle Furchen sowohl der Oberseite als auch der Unterseite jeder Ixodidengattung zukommen, bei einzelnen Arten deutlich, bei anderen stark rückgebildet, manchmal auch fehlend. Die Argasiden hingegen lassen in puncto Furchenverlauf nicht die geringste Ähnlichkeit mit den bei den Ixodiden zu beobachtenden Verhältnissen erkennen. Zu dem gleichen Ergebnis gelangt man auch bei den übrigen Merkmalen. In vieler Hinsicht zeigen die Argasiden aber grosse Ähnlichkeit mit den Uropodiden, von denen sie wohl nach dem Vorgange von P. Schulze abgeleitet werden müssen. Besonders ist in diesem Zusammenhange auf die Milbengattung *Discopoma* hinzuweisen.

Bei einem weiteren Merkmale, den Analplatten, zeigt sich, dass die in der Gattung *Amblyomma* zu beobachtenden Bildungen gar nichts mit denen der Gattung *Hyalomma* zu tun haben, sondern sehr den Analplatten der Rhipicephalusarten gleichen. Auch kommen nur in den beiden letzten Gattungen merkwürdige, von P. Schulze "Peltae" benannte Chitinblättchen vor. Weiter wurden die Palpen, die Proportionen der einzelnen Palpenglieder zueinander, ihre Borstenformeln, die Lage und Gestalt der mannigfachen Palpenanhänge, die Form des Kapitulums, die Anordnung der Zeichnungselemente, die Stigmata und die Augen, die Randschildchen und die Schwanzanhänge genauer untersucht. Auch geographische und biologische Eigentümlichkeiten sind mit berücksichtigt worden.

Im zweiten Abschnitte sind obige Befunde für die einzelnen Zeckengattungen zusammengefasst, und es ergibt sich dabei, dass *Ixodes* Latr. von den übrigen Ixodidengattungen nicht so scharf zu trennen ist, wie etwa diese von den Argasiden. Ferner stellte sich bei eingehender Betrachtung heraus, dass die Gattung *Margaropus* keine verwandtschaftlichen Beziehungen zu Rhipicephalusarten zeigt, wohl aber in vielen Punkten mit *Dermacentor* übereinstimmt. Amblyommaarten, besonders *A. longirostre* Koch, machen in Bezug auf die Form des Kapitulums und die Gestalt der Palpen eine der Gattung *Ixodes* benachbarte Stellung im System sehr wahrscheinlich. Nur in diesen beiden Gattungen tritt zwischen dem Rostrum und den Palpen eine merkwürdige, grössere Lücke auf, die ich kurz Palpenwinkel benenne. Bereits Koch und manch anderer nach ihm haben ihn richtig auf ihren Abbildungen dargestellt, aber nie im Texte erwähnt. Deutlich ist der Winkel noch bei *Dermacentor*-Nymphen zu erkennen; diese Gattung schliesst sich in vieler Hinsicht eng an *Amblyomma* an. Während auch die dickpalpigen Ixodesarten einen ausserordentlichen auffälligen Palpenwinkel besitzen, fehlt er den durchgängig dickpalpigen, ausgewachsenen *Dermacentor* vollkommen. Die Bestimmung der Gattungen bot bislang einige Schwierigkeiten, besonders wenn man nur weibliche Exemplare hatte und keine ♂♂ zur Verfügung standen. Gattungsverwechselungen sind deshalb sehr häufig. Nach Neumann, Ixodidae (*Das Tierreich*, 1911), kann man, wenn z. B. nur ein ♀ vorliegt, die Gattung nicht bestimmen. Die Angaben "palpes longs," "palpes courts" sind doch ausser-

ordentlich relativ und gar nicht einmal für alle Arten einer Gattung gültig. Ein praktisch brauchbares und bequemes Mittel, die Gattung festzustellen, erkannte ich jedoch an der Beborstung der Palpenunterseite. Man legt die betreffende Zecke auf den Rücken und schiebt den Kopf unter das Mikroskop. Schon bei schwacher Vergrösserung lassen sich auf diese Weise *Amblyomma*- und *Hyalomma*-Weibchen leicht voneinander unterscheiden. Bei der Betrachtung der Palpen u n t e r s e i t e stellte sich nun heraus, dass die Anzahl der Borsten an jedem Palpengliede bei *Hyalomma* genau die gleiche ist wie bei *Rhipicephalus*, ja sogar einen ventralen Anhang besitzen beide Gattungen am ersten Palpengliede, der *Amblyomma* in dieser Weise fehlt. Der einzige Unterschied zwischen jenen beiden Gattungen besteht nur darin, dass die Palpen von *Rhipicephalus* kürzer, gedrungener sind als die von *Hyalomma*, und *Rhipicephalus* gefiederte Borsten trägt; bei *Hyalomma* sind sie glatt. Die Borstenformel für *Rhipicephalus* und *Hyalomma*, ist $\frac{2}{7}$ (die unterste Zahl gibt die Anzahl der Borsten am ersten Gliede an, die darüber die des nächsten und so fort), für *Amblyomma* $\frac{3}{4}$. Eine Verwechselung beider Gattungen dürfte daher in Zukunft wohl nicht mehr möglich sein.

Im dritten Abschnitte der Arbeit werden kurz die bisherigen Systeme besprochen (eine eingehende Darstellung findet sich in Nuttall, Warburton, Cooper and Robinson, *Ticks, A Monograph of the Ixodoidea*, Cambridge, 1908–11) and die Ergebnisse der Untersuchung zu einem neuen, natürlichen System zusammengefasst. Interessant ist dabei, dass Lahille 1905 bereits *Hyalomma* von *Amblyomma* trennte; er fasste aber die Gattungen nur zusammen und gab noch keine Richtung für den vermutlichen Aufbau seines Stammbaumes an, in den sich eine ganze Menge Irrtümer an anderen Stellen eingeschlichen haben. 1907 vereinigte aber Warburton *Hyalomma* wieder mit *Amblyomma* als Longirostrata, was von allen folgenden Bearbeitern beibehalten wird. Nuttall und seine Mitarbeiter (1911, p. 209) stellen Neumanns Ansicht (1904) nun in folgendem Schema dar:



Die englische Autoren trennen den ringförmigen Zusammenhang der vier Gattungen *Dermacentor*, *Amblyomma*, *Hyalomma* und *Rhipicephalus* zwischen den beiden letzten Gattungen. Durch meine Arbeit glaube ich aber nachgewiesen zu haben, dass der Ring zwischen *Hyalomma* und *Amblyomma* unterbrochen werden muss. Diese beiden Gattungen haben nur "lange Palpen" gemeinsam, die aber in der Beborstung, den Gliederproportionen und den Anhängen keine Übereinstimmung erkennen lassen, ebenso in den anderen untersuchten Merkmalen.

Am Schlusse der Arbeit habe ich zur schnellen und bequemen Orientierung noch eine kurze tabellarische Übersicht über das Auftreten einiger Merkmale bei den verschiedenen Ixodidengattungen hinzugefügt.

Bei der Einteilung der Ixodiden sind im wesentlichen die von Neumann 1911 aufgestellten Stämme beibehalten, nur ist eine andere Reihenfolge derselben aufgestellt, damit der Name "natürliches System" auch zu Recht besteht. Innerhalb der Stämme ist *Boophilus* Curtice bei den Rhipicephalarien verblieben, *Margaropus* Karsch aber in die Nähe von *Dermacentor* Koch, also zu den Amblyommatrien gezogen worden. Die nähere Begründung hierfür und weshalb ich das Zeckenreich nach dem Vorgange von P. Schulze aus zwei Wurzeln, also diphyletisch ableite, empfehle ich in der Arbeit selbst nachzulesen, die in der *Zeitschrift für wissenschaftliche Biologie* Abt. A. erscheinen wird.

OBELISCOIDES, A NEW NAME FOR THE NEMATODE GENUS *OBELISCUS*.

By H. W. GRAYBILL, D.V.M.

University of California.

In *Parasitology*, vol. xv, p. 340 (20. ix. 1923), I described a genus of nematodes from the rabbit for which I proposed the name *Obeliscus*. Dr Paul Bartsch, of the Smithsonian Institution, has called my attention to the fact that this name is preoccupied, having previously been used for genera of Mollusca by Humphreys in 1797, by Agassiz in 1837, and by Beck in 1837. I therefore propose the new name *Obeliscoides*, type *cuniculi*, to replace *Obeliscus* Graybill, 1923.

ON A CASE OF INTESTINAL MYIASIS IN MAN PRODUCED BY THE LARVAE OF A SARCOPHAGINE FLY.

By D. KEILIN, Sc.D.,
Beit Memorial Research Fellow.

From the Molteno Institute for Research in Parasitology, University of Cambridge.

(With 2 Text-figures.)

The larvae described in this paper were obtained in 1916 by Major C. E. Southon, I.M.S., from a case of intestinal myiasis observed by him in Nairobi (British East Africa). These larvae were sent to Professor J. H. Ashworth and referred to me by Dr G. A. K. Marshall of the Imperial Bureau of Entomology.

The following are the particulars drawn from Major Southon's letter. "From Sepoy, aged 30, who was noticed to be getting anaemic, with loss of appetite. *Anchylostoma* suspected, so patient was starved for 3 days, being allowed water only. On morning of 4th he received

Ol. eucalypti	1 dram
Chloroform	45 minims
Ol. ricini	1 ounce

An hour later repeated half a dose of above, and another half dose again an hour later. Stools washed and strained, 40-50 small larvae were found, but assistant destroyed most of them. (Those sent are the only specimens available so far as I know). Treatment repeated a week later but no more larvae were obtained. The man made a rapid recovery."

An examination of the larvae showed that they belong to a Sarcophagine fly and were all in the first larval stage. The larva when fully extended is 2·3 mm. long. The anterior end is pointed, while the posterior is truncated. The body is composed of a small pseudocephalon, three thoracic and eight abdominal segments. Each segment bears near both the anterior and posterior borders several rows of small, fine hairs. The head, as in all Cyclorhaphous Dipterous larvae, is divided by means of a median fold into two lobes, each bearing an antenna, maxillary palp and a band of vertical narrow chitinous ridges (*l.r.*) extending from the buccal depression to the lateral sides of the lobes.

The buccopharyngeal armature (Fig. 1) is composed of two lateral hooks (*l.h.*), a short intermediate portion (*i*) fused with the pharyngeal or basal sclerite (*ph.*). The latter has the structure typical of all Cyclorhaphous Diptera. The ventral wall of this sclerite shows a series of oblique ridges (*r.*) projecting

into the pharyngeal lumen. Ventrally to the anterior portion of the intermediate sclerite lies the hypopharyngeal plate (*hy.*), into which the common duct of the salivary glands opens. The respiratory system is metapneustic (Fig. 2), *i.e.*, having only one pair of functional spiracles (*s.*), lying in a deep cup-like depression (*d.*) on the dorsal side of the last abdominal segment. The lips of this depression are bordered with a fringe of long and fine hairs.

The presence of two lateral buccal hooks, the metapneustic respiratory system and the presence of the post-abdominal spiracular depression are the main characters which indicate that we are dealing with the first stage larva of a Sarcophagine fly. The presence of pharyngeal ridges shows that the larva

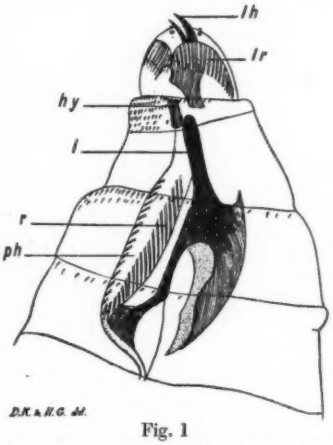


Fig. 1

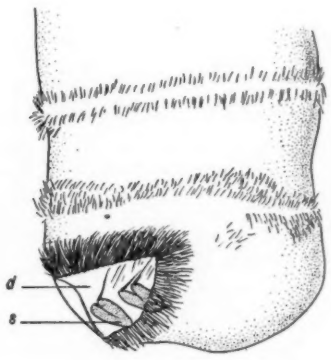


Fig. 2

Text-fig. 1. *Sarcophaga* sp.; side view of the head and buccopharyngeal armature of the 1st stage larva; *i*, intermediate sclerite; *hy*, hypopharyngeal sclerite; *l.h.*, lateral buccal hooks; *l.r.*, rows of lateral ridges; *ph.*, pharyngeal or basal portion; *r*, pharyngeal ridges. × 65.

Text-fig. 2. *Sarcophaga* sp.; side view of the two last abdominal segments of the 1st stage larva, showing the post-abdominal cup-like depression, *d*, and the post-abdominal spiracles, *s*. × 65.

is of saprophagous habit (*i.e.*, feeds usually upon decomposed animal or vegetable substance).

Considering that the larvae of Sarcophagids in their natural habitat grow very rapidly and that the first moult usually takes place within 12 to 24 hours, it must be assumed that the infection took place through eating food containing recently deposited larvae.

The conditions of life in the intestine of man were evidently not favourable for these larvae, as during a period of at least four days they showed no sign of growth. They remained alive, however, and the trouble they caused was probably due to the scratching of the walls of the intestine with their strong buccal hooks.

Several cases of intestinal myiasis due to Sarcophagines have been already recorded by previous authors, but in only a few cases did the larvae succeed

in reaching their full size, so as to be ready to pupate after evacuation. Joseph (1887) mentions, for instance, that while the larvae of *Sarcophaga carnaria* do not grow in the intestine, those of *S. haemorrhoidalis*, on the contrary, live there and grow as parasites, finally reaching their full size.

Joseph's statement concerning *S. haemorrhoidalis* was recently corroborated by two interesting observations related by Aldrich (1916) in his important monographic study on "*Sarcophaga* and Allies in North America." The first instance deals with specimens of this fly received by him from Mr J. B. Gill of the Bureau of Entomology and accompanied by the following note: "Passed a number of rather large dipterous larvae with my stool on the morning of June 21, 1916, after taking a strong dose of 'Pluto' water. Some faeces containing a few larvae caged for the purpose of rearing adults. June 24th, all larvae have left the excrement and entered the sand for pupation. Found five pupae and two larvae buried in the sand in depths varying from 2 to 4 inches. July 3rd, six adults (Sarcophagids) emerged. July 4–16, two adults emerged...."

The second case deals with a batch of adults of *S. haemorrhoidalis* received from Prof. L. Haseman and accompanied with the following information from Dr Al. Lewis.

"The larvae of flies I sent you were collected by the mother from a chamber; I personally do not know that they were passed by the patient. There are four in the family. The father, aged about 30, has never noticed any larvae. The mother, aged about 26, has passed larvae in 1912–13–14–15, none yet this year; has had from one to three attacks each year, in the months of July, August, and September. A girl, aged $7\frac{1}{2}$, first passed larvae in July this year,—flies sent you. Boy aged 5, no larvae this year, one attack last year.... The attacks are short, one or two days; some elevation of temperature (101 to 102 degrees), colicky pains in abdomen, anorexia, sometimes nausea and vomiting. Calomel with salt or oil will bring the larvae, after which the patient recovers."

These two cases show clearly that the larvae of at least one species of this family, namely those of *Sarcophaga haemorrhoidalis*, find in the alimentary tract of man conditions favourable for feeding and growth. They do not seem, however, to live as parasites, but probably feed upon partly digested and decomposed food, which they find in the intestine.

The interesting feature of this case of myiasis is the peculiar capacity of the larvae (*S. haemorrhoidalis*) to live in the media devoid of or very poor in free oxygen, having at the same time their respiratory system highly specialised for the aerobic mode of life.

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ON A NEW CILIATE, *CRYPTOCHILUM BOREALE* NOV. SP., FROM THE INTESTINE OF *ECHINUS ESCULENTUS* LINN., TOGETHER WITH SOME NOTES ON THE CILIATES OF ECHINOIDS.

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(With 3 Text-figures.)

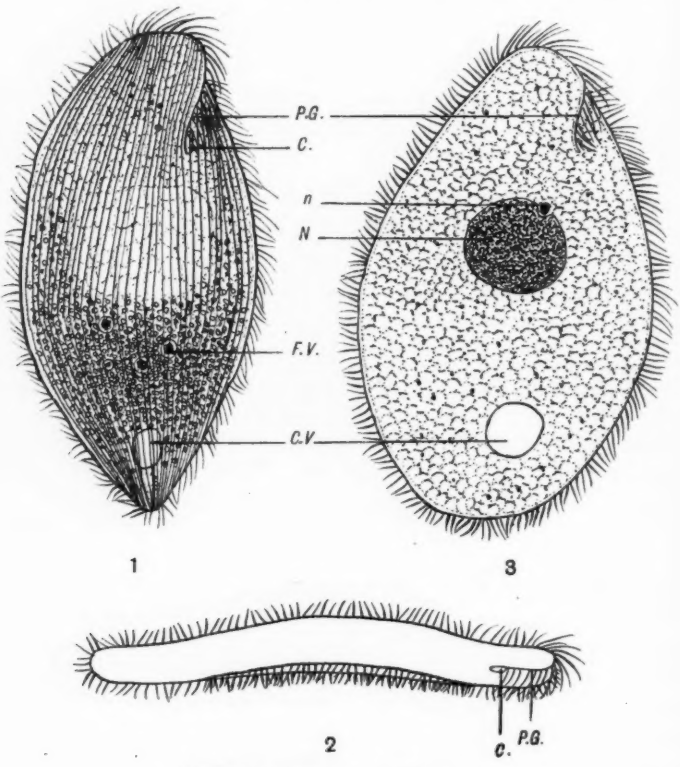
While collecting in the Shetland Islands during August 1923, I made examinations of many marine animals in the search for protozoan parasites. Among the species examined was *Echinus esculentus*, and I was interested to note the presence of a large number of ciliates in the intestine of three specimens. I can find no description of this ciliate, and am obliged to believe that it has never been recorded before. This is somewhat surprising, since many of these sea-urchins must be examined every year in zoological laboratories; it is difficult to understand how such conspicuous parasites have escaped observation for so long.

It is true that a number of ciliates have been recorded from time to time as parasites or commensals of the Echinoidea, mainly from the Mediterranean, but the descriptions of none of these will apply to the ciliate I found. I have therefore decided to name it *Cryptochilum boreale* nov. sp., owing to its seemingly northern distribution.

Of the three specimens of *Echinus esculentus* examined at Fetlar, an island in the Shetlands, all were heavily infected. Unfortunately I was unable to obtain further material owing to excessively stormy weather just prior to my return south; but judging from the richness of the infection found, this parasite would appear to be abundant in the *Echinus* of the district. I thought it possible that infection with *Cryptochilum boreale* might be more or less a local matter, and if so, this might explain why it has escaped notice for so long. Accordingly, I have since examined *Echinus* from various other sources, with the following result:—five *Echinus esculentus* from Millport yielded one very light infection, only two or three ciliates being found; of nineteen from Aberdeen, six were found to contain the parasite, but not in such abundance as those from the Shetlands; twenty-five *Echinus miliaris* from West Mersea, Essex, were totally uninfected, and the same applies to an equal number of *E. esculentus* examined at Plymouth. From this it looks as though *Cryptochilum boreale* may be a northern form.

CRYPTOCHILUM BOREALE.

Cryptochilum boreale was found plentifully in the anterior and middle part of the alimentary canal of the sea-urchin, *Echinus esculentus*. It was particularly common in the sagging loops of the intestine between the supporting mesenteries. None were found among the faecal pellets towards the anus,



Cryptochilum boreale. × 650 approx.

Fig 1. The living ciliate. Fig. 2 The same, seen edge on. Fig. 3. A stained ciliate (Ehrlich's haematoxylin). *C.* cytostome; *C.V.* contractile vacuole; *F.V.* food vacuole; *N.* meganucleus; *n.* micronucleus; *P.G.* peristomial groove.

unlike *Colpidium echini*, described by Russo (1914) as occurring "per più abbondante nella porzione terminale o anale dell' intestino della *Strongylocentrotus lividus*."

In shape, *Cryptochilum boreale* is ovoid and almost as flattened and leaf-like as a *Stylonychia*. One border is rather more convex than the other. At the anterior end of the more concave side lies the small and inconspicuous mouth, overhung by a small flattened lobe. Since convention dictates that the side

on which the mouth is situated shall be called ventral, we must say that the animal is flattened from side to side. It moves usually with the oral (ventral) border to the right, and it is in this position that most specimens are found when fixed; but it appears to be able to move about on either side with equal ease. It swims very actively when placed on a slide, making its way in a rather determined-looking manner through the intestinal contents. It progresses for the most part in a slightly curved course, which is undoubtedly due to the different curvatures of the two edges of the body. As it turns over, it is possible to see that the left hand (lower) surface is slightly concave, and that there is a corresponding convexity on the right hand (upper) surface, which gives the body a humped appearance when viewed edge on.

Specimens vary in size from 115 μ to 140 μ in length, and from 75 μ to 95 μ in breadth.

During life the animals have a rather greyish appearance, the colour being due to the nature of the cytoplasm, which is very granular, with a number of small vacuoles, together with a large quantity of globular, refringent bodies. Both vacuoles and refringent bodies are more numerous in the hinder part of the body; the latter are, however, dissolved in the process of fixation and staining, so that in permanent preparations the cytoplasm has a clear, vacuolated appearance. The ectoplasm is well defined but narrow, being about 2 μ in thickness.

A few small food vacuoles were noted in some of the specimens, situated close together in the hinder part of the body among the refringent bodies, just posterior to the meganucleus.

The cilia are evenly distributed all over the surface of the body. They are fine and are slightly longer in the anterior and oral regions, varying in length on an average from about 3 μ in the posterior part to about 8 μ in the anterior. There is, however, no sharp line of demarcation between the two grades of cilia, but they merely become gradually longer as we follow them from the posterior to the anterior end of the body. The longer cilia in the anterior region sweep across towards the mouth in a very well-marked manner.

The cilia are arranged in longitudinal grooves, running from end to end of the body, which gives the animal a very definitely striated appearance. The number of striations is apparently constant, being 34 or 35 on each surface.

In the extreme posterior part of the body is to be found a conspicuous contractile vacuole. In position it is not exactly in the middle line, but lies slightly towards the oral surface. In shape it is, as a rule, not quite spherical, but takes the form of an irregular ellipse. The expansion of the vacuole is very slow, and it is barely possible to observe its enlargement; the contraction, on the other hand, is sudden and complete, almost all sign of the vacuole disappearing for some little time after the systole has taken place. At the climax of its diastole, the contractile vacuole reaches a size of about 15–20 μ in diameter.

Quite close to the anterior end of the more concave surface, lies a slight depression. This leads to the peristome, which is a definite S-shaped groove running down to the cytostome, which is an oval aperture about 5 μ in length. The whole structure is not very well marked, and though visible in the living specimens, in some fixed and stained examples it cannot be made out or only with difficulty. No undulating membrane has been observed.

The large subspherical meganucleus is situated in the centre of the body. In diameter, it is on an average 20 μ. Its position can very readily be made out in the living animal as a somewhat circular, transparent patch; this transparency is due to the almost complete absence of any refringent bodies in this neighbourhood. From this it may be inferred that the meganucleus extends across the whole thickness from the right hand (upper) to the left hand (lower) surfaces, leaving only a thin layer of cytoplasm on either side. Its appearance, when stained[1], is that of a mass of very darkly stained granules, forming a complex and rather variably shaped network, the meshes of which are sometimes more or less spherical, sometimes rather elliptical. The meganucleus is surrounded by a fine nuclear membrane.

The micronucleus lies close to the meganucleus, and is a small, compact body about 4 μ in diameter. Its usual position in the "resting" stage between nuclear divisions is in a small niche in the side of the meganucleus, similar to what may commonly be seen in ciliates such as *Paramecium caudatum*. This niche lies on the anterior side of the meganucleus, and the micronucleus when lying in it, appears to be more or less spherical. When division is about to take place, the micronucleus apparently comes out of its resting-place in the niche, and moves round to the side of the meganucleus, at the same time becoming ovoid.

The division of *Cryptochilum boreale* exhibits no remarkable features. First of all the micronucleus divides by mitosis, and the two daughter micronuclei pass towards opposite poles of the meganucleus which becomes dumb-bell shaped. Constriction of the cytoplasm then occurs, and two daughter individuals are ultimately produced by transverse fission. It is probable that in the Shetland specimens, the ciliates were undergoing rapid multiplication, for the micronuclei of a large number of individuals appear to be in a condition just prior to, or in the early stages of, mitosis, which might account for the heavy infections found there. Up to the present neither encystment nor conjugation has been observed.

SYSTEMATIC POSITION.

The ciliate I have described belongs to the hymenostomatous Holotricha, and to the family of the Isotrichidae. I have had great difficulty, however, in deciding whether it should be placed as a new species of one of the existing genera of that family, or whether it is not sufficiently different to warrant a

[1] The specimens were fixed in Bouin's fluid, and stained either with Ehrlich's haematoxylin, Heidenhain's iron haematoxylin, or Mayer's acid haemalum counter-stained with eosin.

new genus. In appearance it comes nearest to *Conchophthirus* Stein and to *Cryptochilum* Maupas. *Conchophthirus* was founded in 1861 for a ciliate from the intestine of the gastropod mollusc *Paludina*, other species of which are to be found on the mantle of *Anodonta* and *Unio*. *Conchophthirus* is not unknown as a parasite of the Echinodermata, André (1910) having described a new species from the intestine of *Antedon*.

I have examined *Conchophthirus*, and find that it differs from the ciliate from *Echinus esculentus* in rather important points; thus, the mouth is much larger and of a characteristic funnel shape, and is generally situated in the middle of the ventral edge; the contractile vacuole is placed near the centrally seated nucleus about halfway up the body near the oral surface; the cilia are longer than in the ciliate under discussion, and the striations are not so conspicuous and at the anterior end curve over towards the oral side, in front of the mouth.

My ciliate also differs in a number of points from the ciliate *Cryptochilum echini*, described from *Strongylocentrotus lividus* by Maupas (1883). The mouth of *C. echini* appears to be a median groove formed by the inturned edges of the ventral surface; the number of striations on each surface is 16–18 against 34–35 in the ciliate described above; moreover, *C. echini* possesses a characteristic tuft of elongated cilia at the posterior end of the body, whereas I have not been able to observe the presence of such a structure in the ciliate from the *Echinus*. *C. echini* seems also to be confined to the Mediterranean, if we may judge from the records.

Without having had an opportunity of examining the ciliate from *Strongylocentrotus*, I feel unable to be certain how much importance to attach to these differences. Further acquaintance with the ciliates of Echinoids may justify the creation of a new genus, but in the meanwhile I think it wise to place it provisionally in the genus *Cryptochilum*, as *C. boreale*, taking *C. echini* as the type-species of the genus (*vide infra*, p. 326).

In my search after the scanty references to the ciliates living in Echinoids, I found that they are scattered for the most part in rather inaccessible Italian journals, and, as an aid to other investigators, I have thought it worth while to append here brief descriptions of the various species. It seems probable that some of the finds (*e.g. Euplotes* sp.) were not true parasites or commensals, but bacteria-devouring ciliates from the outside that make their way into the bodies of dying sea-urchins. I am the more inclined to this view, since at Plymouth I myself noticed on several occasions in the perivisceral fluid of moribund *Echinus*, ciliates (? *Paramecium marinum*), which were also to be found free-living on the sides of the tanks and in large numbers among decaying animal matter such as decomposing *Crepidula*.

The earliest mention of ciliates in Echinoderms appears to be that of Hoffmann (1871), who records the presence of ciliates from Echinoids from the Mediterranean. "Der Inhalt des Darmes ist dagegen überaus reich an In-

fusorien, besonders Infusoria ciliata. Dieselbe Arten welche man im Inhalt des Darmkanals findet, kommen auch in der Flüssigkeit der Leibeshöhle, so wie im Blut- und Wassergefässsysteme vor. Ihre Anzahl war zuwielen so gross, das sie die Untersuchung belästigten."

Anophrys echini Di Mauro.

This ciliate is described by Di Mauro (1904) as occurring in the intestine of *Strongylocentrotus lividus* and *Sphaerechinus granularis* amongst the faeces, being particularly abundant in the residue of algae, especially that of *Ulva lactuca*. It is subcylindrical in shape, and varies from 50 μ to 150 μ in length, and 10 μ to 40 μ in breadth, with cilia arranged in longitudinal striations, the number of which is not stated. There is no terminal tuft of cilia. In the neighbourhood of the cytostome, which is an oblong aperture situated about a third of the way from the anterior end of the body, there is a group of large cilia for driving food into the mouth. The pharynx is horseshoe-shaped. The contractile vacuole is posteriorly placed. The meganucleus is spherical and centrally situated, while the micronucleus is very small and lies away from the meganucleus towards the anterior end of the body.

Russo (1914) has noted the presence of a thin membrane in the oral region, and raises the question as to whether this form is not nearer to the genus *Lembus* than to *Anophrys*.

Colpidium echini Russo.

This form has been described by Russo (1914) from the terminal part of the intestine of *Strongylocentrotus lividus*, obtained presumably in the neighbourhood of Catania. It is a small kidney-shaped organism about 65–75 μ in length by 30–35 μ in breadth. The cilia are arranged along 10–12 longitudinal striations. The cytostome is situated some distance from the anterior end, and is apparently large and conspicuous. In front of the cytostome, the body is rounded into a slight prominence that is rather a characteristic feature of the genus *Colpidium*. There is no undulating membrane. The meganucleus is large and lies on a level with the mouth. The micronucleus holds a position anterior to that of the meganucleus.

Cryptochilum echini Maupas.

This species was first described by Maupas in 1883, when he founded the genus. Since that time there has been a considerable amount of discussion as to whether the genus *Cryptochilum* is not synonymous with *Uronema*, in which all the species have been placed by Bütschli. Russo in 1914 has reopened the question, and has put forward cogent reasons for retaining *Cryptochilum* only for the species found in *Strongylocentrotus lividus* and *Sphaerechinus granularis*. The rest of Maupas' species, *C. nigricans*, *C. elegans*, *C. griseolum* and *C. tortum*, all of which are free-living, are probably rightly to be included in the genus

Uronema. Russo points out that the body of *Uronema* is subcylindrical, whereas that of *Cryptochilum echini* is leaf-like ("fogliare") and flattened, and that the single terminal cilium that is characteristic of *Uronema* is not to be found in *Cryptochilum*, which possesses instead a tuft of cilia ("un ciuffo di peli") on a short prolongation of the body.

According to Maupas, *Cryptochilum echini* is a very much flattened form about 70–140 μ in length. The cilia, which are longer anterior to the mouth are arranged in longitudinal rows, the number of which is 16–18. The mouth is situated in the re-entrant angle formed by the junction of the two curves of the ventral edge, rather similar to what is found in *Colpoda*. The contractile vacuole is posterior and ventral, and pulsates with rapid systole and slow diastole. The mega- and micronuclei lie in close apposition in the centre of the body.

The conjugation and life-cycle of *Cryptochilum echini* have been worked out in great detail by Russo and Di Mauro (1905, 1905 *a*, 1905 *b*) and by Russo (1914 *a*, 1916).

Maupas considered this to be a Mediterranean form, in view of the fact that he had noticed it on the coast of Algiers and at Banyuls-sur-Mer, but not at Roscoff, while Russo more recently made his observations from Catania. It is possible that these may be the ciliates referred to by Hoffmann. André, however, in 1910, records the abundant occurrence of what he calls *Cryptochilum echini* from the intestine and perivisceral fluid of *Paracentrotus lividus* and *Echinus miliaris* from Roscoff. In *P. lividus* he describes it as invading the testes but not the ovaries. These ciliates of André's are unfortunately not figured or described, and it is possible that they may be identical with the form described above by me from *Echinus esculentus* in Scottish waters.

Lacrymaria catinensis Russo.

Russo (1914) mentions the occurrence of a new species of *Lacrymaria* from the intestine of *Strongylocentrotus lividus*. He remarks on the comparative rarity of this species, saying that it has only been observed in the months of February and March. He reserves his description of this ciliate for a later occasion.

Euplotes charon O. F. M.

André (1910) records the occurrence of this common ciliate on the exterior and in the perivisceral fluid of *Echinus esculentus* at Roscoff. It is difficult to believe that this "infection" is not accidental, due either to the chance knocking in from the exterior in opening the sea-urchin, or to the fact that the host was moribund. The latter seems the more probable, since he records that they were abundant in the perivisceral fluid. The presence of *Euplotes* on the exterior is scarcely remarkable.

To this list may now be added *Cryptochilum boreale*.

Cryptochilum boreale nov. sp.

Flattened holotrichous ciliate, with a very small mouth near the anterior end; no undulating membrane; striations about 35 on each surface, running from anterior to posterior end; cilia 3 μ to 8 μ long; meganucleus in the middle of the body, ovoid to spherical; micronucleus in a niche in the anterior border thereof; posterior half of the cytoplasm filled with numerous refringent bodies; contractile vacuole conspicuous and at the extreme posterior end.

Dimensions: 115–140 μ by 75–95 μ.

Habitat: Anterior and middle part of the intestine of *Echinus esculentus* from Scotland (Fetlar, Aberdeen, Millport).

In conclusion, I wish to express my thanks to Dr D. L. Mackinnon of King's College, London, for her kind help and advice.

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A REFERENCE LIST OF THE TREMATODE PARASITES OF BRITISH REPTILES.

By WILLIAM NICOLL, M.A., D.Sc., M.D.

The publication of this list of Reptilian Trematode parasites involves little comment. Reptiles form at most an insignificant and unobtrusive part of the fauna of this country and, apparently, few if any, investigations have ever been made of the Trematode parasites which may occur in them. I have personally examined a few snakes but with entirely negative results.

Such records as are available are chiefly due to the investigations of continental observers and the following list is published mainly as an up-to-date summary of that work. It differs only in minor degree from the lists published by Stossich (1895) and Lühe (1909) apart from the fact that it furnishes references to the literature which are omitted by these authors.

Our present knowledge of these forms is due largely to the work of von Linstow (1877–85) and Lühe (1900–11), who have revised and extended the observations of earlier workers such as Rudolphi (1819), Dujardin (1845) and Diesing (1850–8).

LIST OF THE TREMATODE PARASITES OF BRITISH REPTILES ARRANGED SYSTEMATICALLY.

A. Distomata.

I. Telorchidae Looss

Telorchis Lühe 1899

1. T. ercolanii (Montic.) — Monticelli 1893, Stossich 1904

Cercorchis Lühe 1900

2. C. nematoides (Mühling) — Mühling 1898, Looss 1899
3. C. aculeatus (Linst.) — v. Linstow 1879, Braun 1901

II. Plagiorchidae Lühe (Lepodermatidae Odhner)

Plagiorchis Lühe 1899 (Lepoderma Looss 1899)

4. P. mentulatus (Rud.) — Kampmann 1894, Mühling 1898

Saphedera Looss 1902

5. S. longicollis (Abildg.) [= Macrodera naja (Rud.)] — Rudolphi 1819, Lühe 1909

Leptophallus Lühe 1909

6. L. nigrovenosus (Bellingham) [= D. signatum Dujard.] — Monticelli 1893, Odhner 1910

III. Dicrocoeliidae Odhner

Brachycoelium Duj. 1845

7. B. salamandrae (Froelich) [= B. crassicolle (Rud.)] — Minot 1878, Looss 1894, Odhner 1910

IV. LECITHODENDRIIDAE

Paradistomum Stossich 1910

8. P. mutabile (Molin)	Lühe 1900, Rizzo 1902, Odhner 1910

V. PROSTHOGONIMIDAE Lühe

Cephalogonimus Poirier 1886

9. C. spec. (Lühe)	Lühe 1911 (p. 85)

Unclassified Species.

10. Distomum acervocalciferum Gast.	Gastaldi 1854, Rizzo 1902
11. D. allostomum Dies.	Diesing 1850, Ercolani 1882, Stossich 1895
12. D. anguis Linst. (larva)	v. Linstow 1885, Braun 1892
13. D. assula Duj.	Dujardin 1845
14. D. cloacicola Lühe	Lühe 1909
15. D. gracillimum Lühe	Lühe 1909
16. D. megaloon Linst.	v. Linstow 1879, Stossich 1895
17. D. signatum Duj.	Dujardin 1845, Kampmann 1894
18. D. viperae Linst.	v. Linstow 1877, Braun 1893

B. MONOSTOMATA.

19. "Monostomum lacertae" Dies.	Diesing 1850, 1858

C. HOLOSTOMATA.

20. Tetracotyle colubri Linst.	v. Linstow 1877, Villot 1898, Faust 1918

LIST OF THE TREMATODE PARASITES OF BRITISH REPTILES, ARRANGED ACCORDING TO THEIR HOSTS.

OPHIDIA

1. *Tropidonotus natrix* Linst. Grass snake

Telorchis ercolanii (Montic.)	Intestine
Cercorchis nematoides (Mühl.)	Intestine
Cercorchis aculeatus (Linst.)	Intestine
Plagiorchis mentulatus (Rud.)	Intestine
Saphedera longicollis (Abildg.)	Lungs
Leptophallus nigrovenosus (Bellingh.)	Oesophagus
Cephalogonimus sp.	Intestine
Distomum acervocalciferum Gast.	Intestine
Distomum allostomum Dies.	Intestine
Distomum assula Duj.	Intestine
Distomum cloacicola Lühe	Cloaca
Distomum gracillimum Lühe	Oesophagus
Distomum signatum Duj.	Intestine
Tetracotyle colubri Linst.	Subcutaneous cysts

2. *Coronella austriaca* Linn. Smooth snake

Nil.

3. *Vipera derus* Linn. Viper

Distomum viperae Linst.	Peritoneum in cysts
Tetracotyle colubri Linst.	Heart in cysts

LACERTILIA

4. *Anguis fragilis* Linn. Slow-worm

Brachycoelium salamandrae (Froel.)	Intestine
Distomum anguis Linst. (larva)	Intestine

LACERTILIA (*continued*)

5. *Lacerta vivipara* Jacq. Common lizard	
Plagiorchis mentulatus (Rud.)	Intestine
6. *Lacerta agilis* Linn. Sand lizard	
Plagiorchis mentulatus (Rud.)	Intestine
Paradistomum mutabile (Molin)	Gall-bladder
Distomum megaloon Linst.	Intestine
"Monostomum lacertae" Dies.	Intestine

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BIOGRAPHICAL NOTES ON BILHARZ, LOOSS AND LÜHE.

By H. A. BAYLIS, M.A., D.Sc.

(With Portrait-plates 30–32.)

(*From the Department of Zoology, British Museum (Natural History).*)

[Continuing the Series begun in *Parasitology*, vol. XIII, p. 398.]

Theodor Bilharz.

1825–1862.

(Portrait-plate 30.)

Bilharz was born on 23 March, 1825, at Sigmaringen on the Danube, where his father held a position connected with the management of the Royal estates. He was the eldest of nine children, of whom four died young. As a boy he attended the local school, where he displayed exceptional diligence and earnestness in his studies. His love of the study of nature showed itself at an early age, for he was a keen collector of plants, insects and minerals, his tastes in this direction being stimulated by constant access to a collection of natural objects formed by his uncle.

In 1843 he entered the University of Freiburg, where he studied natural philosophy under various teachers, and especially anatomy under Arnold. When Arnold, in 1845, was appointed to a Chair at Tübingen, Bilharz followed him to that University, where he began the study of medicine. In 1847 his essay on a subject (the microscopic characters of the blood of invertebrate animals) set by the Medical Faculty was awarded the prize. He qualified in medicine two years later, and returned immediately to Freiburg to attend von Siebold's lectures, and to study the comparative anatomy of the lower animals. Opportunely for Bilharz, in this year (1849) the post of Prosector at the Anatomical Institute became vacant, and he was appointed to it at the beginning of the winter term. He did not occupy it for long, however, for in the next year he was invited to go to Egypt as Assistant to a former teacher of his, Griesinger, who was now General Medical Director there. He waited only to receive the degree of Doctor of Medicine at Tübingen, and arrived in Egypt early in June.

Under Griesinger he worked in Cairo for two years, at the end of which time, Griesinger's contract having expired, Bilharz was suddenly informed that his salary too would cease. However, the German Consul interested himself on his

behalf, and in the end he was allowed to stay. A new post was found for him as *Chef de Clinique* in the surgical division of the hospital, which was under the direction of Professor Reyer. In 1853 there was a reorganisation of the hospital staff, and Bilharz became chief surgeon of a division for internal diseases. Two years later, at the instigation of Reyer, he was appointed Professor at the medical clinic of the Kasr-el-Ainy Medical School. This Chair he exchanged in 1856 for that of Descriptive Anatomy.

In addition to his other duties, Bilharz discharged those of an expert medico-legal adviser to the Government. In 1861 he was once more transferred, from the internal diseases department to that for syphilis and diseases of the skin. In the winter of 1859 he and his younger brother (who was a medical student, and was visiting him in Cairo) accompanied the German Consul on a brief expedition into Upper Egypt. The time, however, was too short for Bilharz to make full use of this opportunity for scientific collecting.

In the spring of 1862 he was presented to the Duke of Saxe-Coburg-Gotha by the latter's physician, Dr Hassenstein. The Duke was planning an expedition to Abyssinia, and it had been arranged that the Duchess and her ladies were to go as far as Massaua, on the shore of the Red Sea, and there await the return of the Duke and the gentlemen of his suite. Bilharz was commanded to accompany the ladies in the capacity of physician. After some hesitation, he agreed to do so. While at Massaua he attended professionally a German lady resident who was suffering from typhus, and had the misfortune to fall ill of that disease. He returned home immediately, but died on May 9th, a few days after reaching Cairo, at the house of his medical friend Lautner, in spite of the latter's assiduous care. His promising career was thus cut off at the early age of 37.

Bilharz was well known only to those who could boast his close friendship, but he was a valued friend to many. He was unmarried, and lived very simply. In the presence of strangers, Brehm says, he was often painfully shy, though he was a man whose wealth of knowledge was capable of adorning any company. No one, probably, had ever been more universally or more sincerely mourned in the European colony in Cairo. Ecker says that even Bilharz' old Arab manservant, Ali, was inconsolable after his death.

It was natural, especially after the teaching and example of von Siebold, that, finding himself attached to a large hospital in such a country as Egypt, where it was part of his duty to perform often as many as three autopsies daily, Bilharz should turn his attention to helminthology. In one of his letters to von Siebold, written very soon after his arrival in Cairo, he wrote: "Was die Helminthen im Allgemeinen, auch die des Menschen betrifft, so glaube ich, dass Aegypten eines der günstigsten Länder für ihre Entwickelung und ihr Studium ist." Helminthology, however, was not his only interest. Indeed, he probably knew more about Egypt, its people and their customs, its fauna and flora, and the anatomy of the negro races, than any of his contemporaries. But he was a man of innate modesty, and considered his

eleven years' collecting and study entirely insufficient to justify the publication of his results. Consequently the number of original papers published by him was very small. The majority of his helminthological notes took the form of letters to von Siebold, who published them in the *Zeitschrift für wissenschaftliche Zoologie* (of which he was joint editor), at first under his own name, but later under that of Bilharz, adding short notes of his own. The first and most important of these communications published by von Siebold included an account of the extreme frequency of many of the commoner human helminths in Egypt—Bilharz adding, with a note of regret, "Die Filaria medinensis kommt leider hier nicht vor." This publication also contained the first account of a blood-fluke—named *Distoma haematobium* by Bilharz—occurring in man; and the first accounts of another human Trematode to which von Siebold gave the name of *Distoma heterophyes*, and of the little Cestode called by Siebold *Taenia nana*. There were also notes on *Ancylostoma duodenale* (already described by Dubini) and on *Pentastoma constrictum* v. Siebold. Bilharz' communications to von Siebold were illustrated by very carefully and skilfully executed drawings, which were reproduced as plates accompanying the published text.

The discovery of the worm now known as *Schistosoma haematobium*, and the elucidation of its connection with pathological conditions long previously known in Egypt, were undoubtedly the most important contributions to science made by Bilharz. Cobbold, in 1859, justly desired to immortalise him by naming the worm *Bilharzia*, but unfortunately Weinland had, in the previous year, already given it the name of *Schistosoma*. Primarily, however, Bilharz was a comparative anatomist, and apart from these helminthological matters his chief work was an important study of the anatomy and histology of the Egyptian "electric eel" (*Malapterurus*), with special reference to the structure of its remarkable electric organ. A preliminary note on this subject was published in 1853, and a fuller account appeared in 1858, both being communicated to scientific societies by his friend Prof. Ecker. Bilharz had also published, in 1852, a description of *Alestes macrolepidotus*, a new fish from the Nile.

In addition to these researches, Bilharz was also greatly interested in craniological and ethnographical problems, and availed himself of every opportunity to study them, although he appears never to have reached conclusions that he considered worthy of publication.

While in Egypt he kept up his connection with the Natural History Society of his old University of Freiburg (in whose Proceedings his work on the electric eel was published), and presented many specimens of interest, collected in Egypt, to the Anatomical and Zoological Institutes of the University.

THEODOR BILHARZ

1825–1862

Arthur Looss.

1861–1923.

(Portrait-plate 31.)

Born at Chemnitz in Saxony on 16 March, 1861, Looss was the son of a manufacturer, Friedrich August Looss. He received his early education at the local high school, entering in 1880 the University of Leipzig, where for four years he studied natural science under Leuckart. In 1885 he graduated as D.Phil., his thesis being on certain Trematodes. But, though the Trematoda ultimately proved the chief study of his life, the subject of the thesis which, in 1889, he presented for his appointment as a *Privatdozent* in the Faculty of Philosophy was the part played by phagocytes in the absorption of the tadpole's tail. In the same year he also received a prize for an essay on the phenomena of degeneration in the animal kingdom. His great interest in parasitology, however, already showed itself when, in 1892, he published a memoir, or rather a small book of 180 pages, on parasitism in the animal kingdom. This essay dealt very fully with many of the general aspects of parasitism and the history of our knowledge of it.

Looss married, in 1891, Elise Lohse, by whom he had no children. In 1893 he paid his first visit to Egypt, but he did not settle there permanently until after a second visit in 1896 (in which year he had been appointed a Professor Extraordinary at Leipzig). He realised the immense possibilities for the study of helminthology in Egypt, and accepted an invitation from the Government to remain in Cairo as Professor of Biology and Parasitology, a post specially created for him in the Egyptian Government School of Medicine. This position he held for eighteen years, until, after the outbreak of war, his presence became, not without reason, undesirable in Cairo, and he was dismissed from Egypt at the end of November, 1914. Like others who were expelled in similar circumstances, he received pecuniary compensation from the Government for such of his belongings as remained in British hands. The helminthological collections that he had been mainly instrumental in forming were left in the School of Medicine. His library, or at least the bulk of it, was returned to him in Germany during 1921 and 1922.

On his return to Germany he went to the house of his brother-in-law at Leipzig, but soon volunteered for military service, and his knowledge of languages made him useful to the German Government. He appears to have served for a time in Belgium as a Captain, but in 1918 he returned to Germany and stayed for a year in Leipzig. In the following year a post as Assistant in the Zoological Institute at Giessen was found for him by Prof. Spengel. For a few terms he lectured and demonstrated there, and devoted his spare time, among other things, to work on a third edition of Mense's *Handbuch der Tropenkrankheiten*, to which he had, in 1905, contributed a section on diseases caused by worms and Arthropods.

In 1921 Looss received the degree of Doctor of Medicine *honoris causa* at Giessen, and in 1922 he became an Honorary Professor in the Faculty of Philosophy. Besides his distinctions in the Fatherland, he was a Foreign Member of the Royal Society of Sciences of Upsala. In his later years he was greatly troubled with asthma, which finally made it impossible for him to continue his scientific work. He died at Giessen, at the age of 62, on 4 May, 1923.

Looss' enthusiasm and energy as a research worker have probably seldom been surpassed, and all his work was characterised by a painstaking attention to detail that is unfortunately rare. Not only was his descriptive work of the highest order, but his descriptions were illustrated by drawings from his own hand of a quality that is unattainable save by the exceptionally gifted. In addition to spending immense care upon every piece of research, Looss was a voluminous writer. Judged by the mere number of his publications, he would not, perhaps, be called one of the most prolific writers among helminthologists. In 25 years, however, he produced some 65 original memoirs, many of which were monographic in scope, and all were important. After the few early excursions into other subjects already referred to, he returned in 1893 to the special study of the Trematodes, publishing in that year a paper on the homology of Laurer's canal, and in the following year an elaborate study of the Trematodes of European fishes and frogs. This was followed by detailed accounts of the anatomy of *Heterophyes* and of *Bilharzia*. On settling in Cairo he began to publish a series of "researches on the parasitic fauna of Egypt," dealing chiefly with Trematodes. His paper on *Heterophyes* was amplified in 1902 into a revision of the genus. By 1899 his researches among the Trematoda enabled him to publish his "Attempt at a natural classification of the genus *Distomum*." This was the first serious attempt to split up the old, unwieldy group collectively known under that name into smaller subdivisions, and may almost be considered the starting-point of the modern systematic conception of the digenetic Trematodes. Regarding "*Distomum*" as representing a family rather than a genus, Looss established nearly sixty new genera in this one monumental paper, the majority of which hold good to the present time.

In 1898 he added the Nematoda to his subjects of research, and for the next ten years was mainly occupied with this group, paying particular attention to the life-history of the Ancylostomes and to the hitherto little-known but, as he was the first to show, extremely numerous Strongylidae of the horse and ass. His preliminary account of his systematic researches on the latter subject was published in the *Centralblatt für Bakteriologie* in 1900, and was followed by the well-known and exhaustive treatise in the *Records of the Egyptian Government School of Medicine* about a year later. Previously only two species of Strongylidae had been recognised as existing in members of the horse tribe. Looss showed that each of these was a collective group, and distinguished altogether no less than eighteen species, falling into four genera.

By the patient and accurate study of minute characters he was able to separate thirteen species of the genus now generally known as *Cylicostomum*, of which only one had previously been described.

In 1898 Looss had published his first notice of the discovery for which he is most famous. Although well known, the story is worth repeating. During some experiments in which he was dropping cultures of the larvae of *Ancylostoma duodenale* into the mouths of guinea-pigs, he accidentally allowed a drop of liquid containing larvae to fall on to the back of his hand. Noticing that this was followed by a burning sensation and reddening of the skin, he deliberately repeated the experiment on himself, and was led to believe that his previous infection with the adult worms had been acquired in the same way. In 1901 he published an account of an experiment designed to prove the fact of skin penetration, in which he had placed larvae on the skin of the leg of a native boy, an hour before it was to be amputated. Subsequent examination of sections from the marked area of the limb revealed larvae in the hair-follicles and also in the epidermis and corium. The conclusion was made still more certain by a further experiment on a native volunteer, who eventually became infected with adult worms. Moreover, by experiments on dogs Looss was able to demonstrate the migration of the larvae of *Ancylostoma caninum* from the skin through the lungs and trachea on their way to the alimentary canal, and gave an account of his results at the sixth International Zoological Congress, held at Berne in 1904.

Looss thus established the fact of percutaneous infection with hookworms, which has changed the whole subsequent course of research and pointed the way for effective preventive measures against one of the worst scourges of tropical countries. He also showed that the behaviour of *Strongyloides stercoralis* was similar to that of the Ancylostomes, and did not hesitate to make use of his own person as a subject of experiment. In 1905 he published the first part of his famous monograph on *Ancylostoma duodenale* in the *Records* of the School of Medicine. Besides being an exhaustive treatise on the anatomy and life-history of this species, this work also included a wealth of information, largely new, on other forms related to it, and became the standard work on the hookworm family. The plates illustrating it, in excellence of draughtsmanship and accuracy of detail, are perhaps as fine a piece of work as any helminthologist has ever produced. The second part of the work, dealing with the developmental history of the larvae during their free-living stage, was not published until 1911.

While working at these problems Looss was also busily collecting material for systematic work, and at the same time perfecting his methods of preserving such material for study. In 1901 he published an account of the methods that he had found in practice to be the most satisfactory, and his researches in this direction have proved of very great benefit to other workers. In particular, he was the originator of the hot-alcohol method of preserving Nematodes, which has now been almost universally adopted. In 1904 he

published a full description, illustrated in his usual detailed manner, of the anatomy of *Filaria loa*, another important human parasite in Africa. In 1907 he returned once more to the intensive study of the Trematodes, on which he published in that year several important systematic papers.

The effect of Looss' work and the weight of his opinion upon all helminthological matters were immense, and, with one possible exception, his influence was entirely beneficial to the advance of knowledge. There was one subject upon which he held with great determination to mistaken views, and it is possible that the discovery of the truth may have been somewhat delayed as the result of his influence. He refused to believe in the existence of an intermediate host for the Schistosomes which are so prevalent and so injurious to man in Egypt, and professed to be convinced that their development was direct. He also challenged vigorously, but without sufficient proof, the view that the terminal-spined and lateral-spined Schistosome eggs met with in Egypt, the former generally found in the urine and the latter in the faeces of the patients, belonged to two distinct species of worms. When, in 1907, the species producing lateral-spined eggs was provisionally named by Sambon *Schistosoma mansoni*, Looss ridiculed the alleged new species, and in 1911 he was still maintaining that the lateral-spined eggs were merely abnormal eggs of *S. haematobium*. But the subsequent discovery by Leiper of specific characters in the adult worms proved that in this matter Looss' judgment was at fault. As regards the life-history, the discovery by Japanese workers that a snail served as the intermediate host of the Japanese Schistosome paved the way to the discovery of the snail-hosts of the two African species, in which Leiper again played the chief part.

The work of Looss stands as an example and an inspiration to those who come after him. His discoveries and methods infused new life into the whole science of helminthology, and his name will be honoured by posterity as one of the greatest in its annals, and one worthy to rank with that of his great teacher, Leuckart.

Maximilian Friedrich Ludwig Lühe.

1870–1916.

(Portrait-plate 32.)

Max Lühe, one of the most brilliant of European parasitologists, was born on 30 April, 1870, at Augustenburg in the island of Alsen, which forms part of Schleswig. His father was an Army surgeon, and his mother's maiden name was Richthofen. After attending various schools (at Stralsund, Belgard, and elsewhere), he passed at the age of 18 into the University of Freiburg, and later went to those of Berlin and Königsberg. He studied Medicine and Natural Science, and in March 1893 sat his qualifying examination in Medicine at Königsberg. In the following October he was appointed Assistant in the Zoological Museum of that University under Professor Max Braun, a post

ARTHUR LOOSS

1861–1923

which he held until Easter, 1913. In December 1894 he took the degree of D.Phil., with a thesis on the morphology of the scolex of *Taenia*. He became a *Privatdozent* at the University in Zoology and Comparative Anatomy in January 1897. In 1909 the title of Professor was conferred upon him, and in 1911 he was appointed Lecturer in Elementary Human Anatomy and Physiology, as well as in Systematic and Faunistic Zoology.

Lühe is said to have been a zealous member of the National Liberal Party and of the East Prussian Aeronautical Society. He married Veronica, the daughter of a Lieutenant-Colonel Schulz, of Berlin. There were no children of the marriage. On the outbreak of the war, Lühe placed himself at the disposal of the German Board of Health, and did medical duty for some time in the military hospitals in Königsberg. Later he volunteered for active service as a surgeon, and was sent to Lithuania, where, while combating typhus, he unfortunately contracted the disease, and died in hospital at Lida on 3 May, 1916, at the comparatively early age of 46.

Lühe was an energetic research worker and a prolific writer. In addition to his teaching and other duties, he found time between the years 1894 and 1912 to publish, in an uninterrupted stream, some sixty original papers, besides a number of reviews, bibliographies and biographical notices. His researches embraced a number of parasitological subjects, and were by no means confined to helminthology, though in the latter field alone he achieved a volume of work which has rarely, if ever, been exceeded in an equal period by any other worker. And not only was his quantitative output great, but it is probably true to say that in quality not one of his contributions to science was unimportant. Most of his work has already stood the test of criticism, and he has left to succeeding generations of helminthologists a legacy of useful and, for the most part, thoroughly sound systematic work which goes far towards making their task an easier one.

At first his purely helminthological work was confined to the Cestoda. During the years 1894 to 1897 he published a series of important papers dealing with the minute anatomy, especially of the nervous and muscular systems, of the Taeniidae and of the Pseudophyllidean Cestodes, including *Ligula*. He did much for the classification of the Cestoda. His studies of the Bothriocephalidae (using this term in its old, wide sense) may be described as pioneer work of no mean order, and his arrangement of these forms is likely to prove a firm foundation for the classification of the future.

In 1899 Lühe began to turn his attention to Trematodes as well as Cestodes. In this year occurred the unfortunate coincidence by which the same genus was named *Lepoderma* by Looss and *Plagiorchis* by Lühe, the two names being actually published on the same day. Unfortunately, as Looss was not slow to point out, Lühe, while indicating the contents of his genus, had omitted to designate a genotype, and, though he subsequently did so, the name proposed by Looss has held the field. From this time onwards Lühe's attention was divided fairly evenly between Cestodes and

Trematodes, and he continued to pour out a steady stream of valuable contributions to our knowledge of the structure and classification of both groups.

In spite of his preoccupation with helminthology, Lühe found time for numerous studies in other directions, chiefly among the Protozoa. In 1895 he was writing on the locomotion of Diatoms and Gregarines, in 1896 on the infection of fishes with Myxosporidia. He also published accounts of the life-histories of Sporozoa (especially the malarial parasites), of the structure and development of Gregarines, and of the occurrence of Trypanosomes and the diseases caused by them; and even a paper criticizing certain supposed cases of parthenogenesis in mosquitoes.

He collaborated with Professor Max Braun in the preparation of the well-known *Handbook of Practical Parasitology*, of which an English translation by Linda Foster was published in 1910.

But of all Lühe's work perhaps that for which he will be most gratefully remembered is the admirable and concise series of handbooks on the parasitic flatworms and Acanthocephala in Brauer's *Süsswasserfauna Deutschlands*, a work to which every helminthologist must make frequent reference. In these little treatises Lühe displayed his genius in its highest form, combining his exceptional power, as a systematist, of seizing upon the essential diagnostic characters of species and genera, with a brevity and directness of style all too uncommon, and for which all who tread the thorny path of helminthology must be genuinely thankful.

The writer of the foregoing notes wishes to express his grateful thanks to Professor Dr Max Braun, of Königsberg, for much information very kindly supplied by him, especially regarding Lühe, who had been his pupil and assistant. Some details as to Lühe were also derived from an obituary notice in *Leopoldina*, Halle, for October 1916. With regard to Bilharz, the fullest information available is contained in a sketch of his life read before the Natural History Society of Freiburg by his friend Ecker in 1862, and published in its Proceedings in 1865. Of this and of an obituary notice by Brehm in *Petermann's Mittheilungen* for 1862 the writer has made free use, as well as of some notes supplied by Professor Braun. For Looss, besides some information sent by Professor Braun, and some derived from other sources, full advantage has been taken of the biographical notice by Fülleborn in the *Archiv für Schiffs- und Tropen-Hygiene* for August 1923.

The accompanying Portraits are from the Collection given by Professor Nuttall to the Molteno Institute: that of Bilharz is from a Portrait at the Cairo School of Medicine of which Professor E. Hindle sent a photograph; that of Looss from a photograph presented by Professor J. W. W. Stephens, of Liverpool; that of Lühe from a photograph purchased in Germany by Professor Nuttall.

MAX LÜHE

1870–1916

NOTES ON THE TAXONOMY AND NATURAL RELATIONSHIPS OF FLEAS, WITH DESCRIPTIONS OF FOUR NEW SPECIES.

By H. E. EWING,

Bureau of Entomology, U.S. Department of Agriculture.

(With Plate XIV and 1 Text-figure.)

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Recently the writer has had occasion to go over and help arrange the United States National Museum collection of fleas. Although not as extensive as some of the other well-known flea collections, it is invaluable because it includes the types of Professor Carl F. Baker, of the University of the Philippines, and of Doctor Carrol Fox, Surgeon, United States Public Health Service, two of the foremost authorities on the Siphonaptera.

When Dr Fox returned to Washington in the spring of 1923, steps were taken to rearrange Baker's types and other specimens of Siphonaptera and distribute them in the museum collection. This entailed some labour since most of the generic names employed by Baker in 1904 had since been changed. With the help of Dr Fox and of Dr E. A. Chapin, of the Bureau of Animal Industry, the writer has rearranged the Baker and Fox specimens in accordance with the recent classifications of Jordan and Rothschild. During this rearrangement notes were made in regard to synonymy, and among the undetermined or wrongly determined material four new species were found. These notes and

the descriptions of the four new species, together with other notes on theories of the natural relationships of the Siphonaptera, are combined in the present paper.

I. The Poultry-Infesting Species of *Ceratophyllus*.

Jordan and Rothschild (1920) in their excellent review of the American bird Ceratophylli have called attention to the particular need of further study of the chicken-infesting species and their near allies. Three species have been reported from chickens in America: (*a*) *C. gallinae* Schrank, long suspected as being a species occasionally reported from fowls in this country; (*b*) *C. niger* Fox, 1908, first described from specimens taken from man and a mouse, but which soon proved to be a common species infesting chickens on the Pacific coasts; (*c*) *C. gibsoni* Fox 1914, taken from a hen-house at Ottawa, Canada. These three will here be considered in the order named.

Ceratophyllus gallinae (Schrank) 1803.

(The Common Hen Flea of Europe.)

SYNONYMY GIVEN IN THIS PAPER:

1896. *Pulex avium* Osborn (in part). *U.S. Dept. Agr., Div. Ent., Bull.* 5, n.s. p. 147.
1920. *Ceratophyllus gallinae* (?) Jordan and Rothschild. *Ectoparasites*, I. 70.

For many years this species was considered as a synonym of *Pulex avium* Taschenberg. In this country Osborn (1896) uses the name *avium* following Taschenberg. He states: "According to Taschenberg, the common fleas affecting a great variety of birds, including the domestic fowls, are all representatives of a single species, and although some of the forms were described as early as 1832 to 1835 he brings them all together, and, discarding the various names referring to particular hosts, as *gallinae* Bouché, *columbae* Gervais, *hirundinis* Sam., *sturni* Dale, and *fringillae* Walk., proposes a new name, *avium* to stand for the species." Following the example of Professor Osborn the present writer published (1913) a figure and notes dealing with our Western hen flea under the name of *avium*. Now a comparison of specimens which I collected at that time with those of *Ceratophyllus niger* Fox has been made by Dr Fox and he pronounces the species his *niger*.

Jordan and Rothschild (1920) mention receiving a specimen taken at Barker, New York, collected by E. P. Felt, which they questionably determine as *C. gallinae* stating: "As it is a ♀ there remains still some doubt that it really is *gallinae*, the ♀♀ of several species running so close that we are as yet not sure how to differentiate them."

Recently Mr M. D. Leonard, Associate State Entomologist of New York State, sent the writer a male and female specimen of the same lot that the Jordan and Rothschild specimen came from. These were found to agree in all respects with specimens of *C. gallinae* from Europe. So nearly alike were the males of the two lots that they agreed even in the minutia of the chaetotaxy

of the claspers. The marginal setae of the movable finger in both individuals were as represented in Pl. XIV, fig. 1. Above there were the three very long subequal setae, then just below a minute one, next a shorter long seta, followed lower down by a yet longer one, then by a very small seta. The different parts of the claspers of the males of the two lots: the base, the manubrium, the process and the movable finger, all corresponded with remarkable agreement.

As a result of these comparisons the old question as to the probable occurrence of *Ceratophyllus gallinae* in North America would seem to be settled. But the Barker, New York record is not the only authentic one.

During this last year (1923) Mr Bishopp received four female specimens, taken in a hen-house at Eliot, Maine, August 21, by M. A. Stewart. These specimens, which Bishopp questionably determined as *C. gallinae*, were sent to the writer for study. They were found to agree in all respects with a specimen from Europe. The receptaculum seminis was slender as in *C. gallinae* though not so slender as it is in specimens of *C. niger*. The number of setae in the pronotal comb of one specimen, cleared with potassium hydroxide, showed twenty-eight teeth. In view of the determination of specimens from both New York and Maine, by direct comparison with specimens from Europe, the occurrence of *C. gallinae* in this country should be considered as definitely established.

Ceratophyllus niger Fox 1908.

(The Western Hen Flea.)

SYNONYMY GIVEN IN THIS PAPER:

1908. *Ceratophyllus niger* Fox. *Ent. News*, XIX. 434.
1913. *Pulex avium* Ewing. *Oreg. Agr. Coll. Bull.* 92, Ext. Ser. II. No. 5, p. 14.
1920. *Ceratophyllus niger* Jordan and Rothschild. *Ectoparasites*, I. 70.

Jordan and Rothschild gave the following records for *niger*: from man, San Francisco, California; in a hen-house and from chicken nests, Tacoma, Washington and Bridgeport, California; from a hen, Essington, British Columbia; from *Planesticus migratorius*, Okanagan Landing, British Columbia. The writer has many specimens of *C. niger* taken from a hen-house, Corvallis, Oregon and four specimens taken by Fox from a sparrow's nest, Fruitvale, California.

C. niger is more nearly related to *C. gallinae* than to *C. gibsoni*. The differences between *niger* and *gallinae* though several and definite are not marked. Probably the best characters for the separation of the two species is to be found by comparing the claspers of the males (Pl. XIV, fig. 1). In *niger* the movable finger (*F*) is much shorter and stouter than in *gallinae*, also the fifth and sixth marginal setae are much shorter, while an enormous discal seta is present which is lacking in *gallinae*. The clasper itself is differently shaped in the two species, particularly is the process (*P*) of *niger* more truncate and tipped with much shorter setae.

Ceratophyllus gibsoni Fox 1914.
(Gibson's Hen Flea.)

SYNONYMY GIVEN IN THIS PAPER:

1914. *Ceratophyllus gibsoni* Fox. *Hyg. Lab.* (*U.S. Public Health Service*), *Bull.* 97, p. 15.
1920. *Ceratophyllus gibsoni* Jordan and Rothschild. *Ectoparasites*, I. 70.

Some uncertainty has existed as to the status of the species described as *gibsoni* by Fox in 1914, from specimens taken in a hen-house, at Ottawa, Canada, by Arthur Gibson, July 13, 1909. Jordan and Rothschild (1920) state that specimens should be compared with *C. garei* Rothschild and *C. gallinae* Schrank. Fortunately the United States National Museum has the type specimens of *gibsoni*, and these have been used by the writer in making this comparison suggested by Jordan and Rothschild.

When the types of *gibsoni* were compared with some specimens of *garei* kindly loaned by Dr Chapin, of the Bureau of Animal Industry, it was observed that the tail of the receptaculum seminis of *garei* is slender, being about three times as long as broad, while in *gibsoni* it is only about one and a half times as long as broad. The head of the receptaculum seminis is also much more slender than in *gibsoni*. The affinities of *C. gibsoni* are rather with *C. niger* and *C. gallinae* than with *garei*. In *garei* the process of the clasper is longer than in any of the chicken infesting species.

When compared with *gallinae* it was found that *C. gibsoni* differed chiefly in the number of setae in the pronotal comb and in the shape of the receptaculum seminis (Pl. XIV, fig. 1). The number of setae in the pronotal comb was found to be 26 and 27 (not 25) for *gibsoni* and 29 for *gallinae*. The receptaculum seminis (Pl. XIV, fig. 1) is short and stout and not at all like that of either *gallinae* or *niger*; however, the type specimens of *gibsoni* are considerably distorted, as if they had been boiled too long in caustic. In view of this condition of the specimens some allowance should be made for the distortion of the receptaculum seminis. Until more records are obtained in the eastern part of North America for chicken infesting Ceratophylli the sinking of this species can hardly be justified.

In order to show both the affinities and differences of the three chicken infesting Ceratophylli a table is given of their characters as follows:

Key to the Chicken Infesting Species of Ceratophyllus.

A. Pronotal comb with twenty-eight or more spines; head of the receptaculum seminis in female but slightly broader than tail but over twice as long.

B. Apical area of metanotum not reduced; finger of clasper of male over three times as long as its greatest width and extending for one-half its length beyond the tip of process of clasper ... ... *C. gallinae* Schk.

BB. Apical area of metanotum considerably reduced; finger of clasper of male about twice as long as its greatest width and extending for about a third of its length beyond the tip of process of clasper ... ... *C. niger* Fox.

AA. Pronotal comb never with more than twenty-seven spines; head of the receptaculum seminis of female almost twice as broad as tail and about one and a half times as long ... ... *C. gibsoni* Fox.

II. The Genus *Odontopsyllus* Baker.

In 1905 Baker established the genus *Odontopsyllus* for four American species of *Ceratophyllus* which he said possessed "the striking Pulex-like character of minute teeth on the inside of hind coxae." Although affinities of the genus with *Pulex* are clearly indicated the group as a whole appears to have been one of questionable unity. Rothschild (1915) made *charlottensis*, one of the four species originally included by Baker in the genus *Odontopsyllus*, the type of a new genus, *Catallagia*. This genus, according to Rothschild, agrees with *Neopsylla* Wagn. in nearly all particulars, but differs in the total absence of genal spines. In establishing this new genus Rothschild pointed out that *Odontopsyllus* Baker was very close to *Ceratophyllus*. He states: "This latter genus, however, is very close to *Ceratophyllus* Curt. (1832) being distinguished from it by the hind coxa bearing a patch of spiniform bristles on the inner surface."

Rothschild made other readjustments in regard to *Neopsylla* Wagner. Into this genus he put *Typhlopsylla setosa* Wagner, *Typhlopsylla grandis* Rothschild and *Ctenophthalmus wemanni* Rothschild. He stated that what Baker described in 1895 as *Pulex gigas* Kirby was clearly related to *Neopsylla grandis* and "possibly identical with it."

The cotypes of *charlottensis* Baker, the type species of *Catallagia* Rothschild, have been examined by the writer and the characters mentioned by Rothschild noted. While there is no genal comb or ctenidium present in this species, there is present at the same position occupied by the two-spined ctenidium *Neopsylla* a dark chitinous thickening (Pl. XIV, fig. 2) which probably represents the ocular tubercle of the degenerate eye of *Neopsylla*. This structure may represent, however, the degenerate remains of the *Neopsylla* type of ctenidium. Ctenidia in the fleas develop, as complete series of intergrades show, from setae. Each tooth or spine originates as a thickened seta, which increases in its thickness in the series of species until a ctenidium is produced. On the other hand degenerate specialization, as it affects ctenidia, acts in a somewhat different way. Spines of a ctenidium disappear usually, if a series is interpreted rightly, by becoming either shorter or smaller, or both. The whole process in the case of the *Neopsylla* group of genera has certainly been in the line of degeneration or reduction in the size or number of ctenidial spines. In the case of *Catallagia* it may be that the chitinous thickening represents the vestige of a spine of the ctenidium; however, if comparison is made with different *Neopsylla* species it will be found that by virtue of both structure and position it would appear to represent the ocular tubercle of the degenerate eye.

In regard to the affinities of *Odontopsyllus* Baker, *sensu stricto*, Rothschild is certainly correct in maintaining that it is very close to *Ceratophyllus* Curtis. An examination of the type specimen of *O. multispinosus* Baker, the type species of the genus *Odontopsyllus*, fails to reveal a single character whereby the genus could be separated from *Ceratophyllus* except for the one given by

Baker and mentioned by Rothschild, *i.e.*, the presence of a patch of spine-like setae on the inside of each of the hind coxae.

Rothschild's suggestion of the possible synonymy of the species described by Baker in 1895 as *Pulex gigas* Kirby with his *Neopsylla grandis* has been considered. A female specimen labelled *Pulex gigas* Kirby by Baker is in the United States National Museum. It was taken from a rabbit by Baker himself at the Michigan Agricultural College, East Lansing, Michigan. This specimen agrees in nearly all particulars with Rothschild's description, but has 22 teeth in the pronotal comb instead of 20. If this specimen were a male so that a comparison could be made with Rothschild's drawing of the genital armature of *grandis* much more certainty as to the synonymy of the two might be adduced. The difference of two spines in a ctenidium with such a large number of spines may well be within the range of individual variation. If this be granted it would appear that the two species should be considered as synonyms, as Rothschild has suggested.

In the same paper in which Rothschild established *Catallagia* he also made another genus in the *Neopsylla* group. This genus, *Phalacropsylla*, was established for a new species, *paradisea*, taken from an *Epimys* sp., *Mus.* sp., and civet cat, in Arizona. In it the frontal tubercle is said to be present, hence in this character a relationship is shown with *Ctenophthalmus*.

Jordan and Rothschild (1915) established the genus *Atyphloceras* for *Ceratophyllus multidentatus* Fox and a closely related species which they described as new. This genus was stated to be similar to *Typhlocerus* Wagner. While examining the types of *multidentatus*, the writer was struck by its remarkable similarity with Baker's *charlottensis*, the type of Rothschild's *Catallagia*. A comparison between the two species showed them to be distinct yet they have remarkable resemblances. Were it not for the presence of two receptacula seminis in *Atyphloceras*, this genus would probably fall into the synonymy of *Catallagia*; but, however, the single difference in the two species in this fundamental character is amply sufficient for their generic distinctness.

The relationships of these genera of the *Neopsylla* group are here shown in the following key:

a^1. Head entire, not divided (at least in the female) into two regions by any dorsal suture or dorsal apodeme above the base of antennae; cephalic ctenidium wanting. **Ceratophyllus** group.

b^1. Hind coxae each with a patch of spine-like setae on the inside. **Odontopsyllus** Baker.

b^2. Hind coxae without patches of spine-like setae on the inside. **Ceratophyllus** Curtis and related genera.

a^2. Head divided into two regions by a dorsal suture, or a dorsal apodeme, or by both a suture and apodeme situated above the bases of the antennae; either a cephalic ctenidium or dark, chitinous area present at the position of ctenidium.

b^1. Cephalic ctenidium present, consisting of two overlapping spines, one being situated above the other.

c^1. Frontal tubercle present ... ... **Neopsylla** Wagner.

c^2. Frontal tubercle wanting ... ... **Phalacropsylla** Rothschild.

b^2. Cephalic ctenidium wanting but a heavily chitinized area in the position of the ctenidium present.

c^1. Female with a single receptaculum seminis ... **Catallagia** Rothschild.

c^2. Female with two receptacula seminis ... **Atyphloceras** Jord. & Roths.

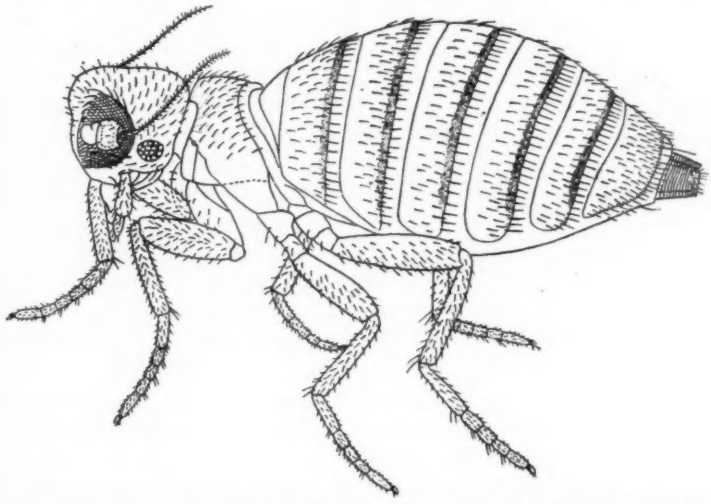
III. Recent and Old Hypotheses as to the Origin of Fleas.

The question of the natural relationships and origin of fleas has been revived by Martini (1922) who advances the view that they should be considered as derivatives of the coleopterous group Staphylinoidea. He would also place them in the order Coleoptera, as a suborder Aphaniptera. Many homologies between fleas and the genus *Oxytelus* are shown, and figures representing cross sections of a flea and an Oxyteline are given in which the fundamental structures are homologized.

Interesting and valuable as this contribution is, yet it should be noted that the homologies which are pointed out by Martini between internal structures of the fleas and Staphylinoidea are of a very general nature as a rule, and also doubtless exist between fleas and many other insects. Probably most entomologists will yet favour some of the older views as to the origin of fleas.

The Hypothesis of Phorid Relationship.

Prominent among the older theories of the origin of fleas is that of Dahl (1898). According to Dahl, fleas and dipterous Phorids have arisen from a



Text-fig. 1. Degenerate dipteron of the family Phoridae, a female of *Chonocephalus buccatus* Malloch, × *ca.* 75.

common hypothetical stemform to which he gives the name *Archiscatopse*. Not only are the Phoridae very degenerate in many respects but some of their adults have developed habits of commensalism very suggestive of parasitism. Among Phorids, types exist also that have a superficial resemblance to fleas (Text-fig. 1). Female Phorids may show a complete loss of wings and halteres; the antennae may be largely concealed in fossae, suggesting

the condition found in fleas; the thorax may be both reduced and compressed; the eyes degenerate; and finally rows of enlarged setae suggestive of the ctenidia of fleas may be developed. Dahl points out several important homologies existing between fleas and Phorids. There are, it appears, no serious difficulties in homologizing the antennae and mouth-parts of the two groups. His interpretation of the degeneration of the Phorid compound eye into the minutely spined area found in some fleas just above the antennal fossa, is certainly very speculative. This structure he figures for the male of *Ctenocephalus canis*. In order to get a better understanding of this structure the writer has made a careful examination of it in this species. In the male of *Ctenocephalus canis* there is a darker or pigmented area situated along most of the upper border of the antennal fossa (Pl. XIV, fig. 3). This area includes about the upper fourth of the fossa itself, the margin of the occiput where the latter joins the fossa, and a little of the area of the occiput next to the margin. The coloration of this area is not due to the deposit of a specific pigment apparently, but to the natural staining of the chitin. It is most pronounced at the central part of the area and fades away gradually toward the periphery. Using the oil immersion lens, the microsetae (or scobinations?) were studied. These structures (Pl. XIV, fig. 3) are minute, spine-like, quite sharp at apex, and are placed into close fitting alveoli, or calices, at their bases. It would appear that they are really small setae instead of being mere scobinations. The number of these minute, spine-like structures is about two dozen but varies somewhat. In *Ctenocephalus canis* they are arranged into two irregular rows.

In the female of *Ctenocephalus canis* and of some other species the dark area above the antennal groove is present, but there are no minute spine-like structures. In many of the flea genera, other than *Ctenocephalus*, the spine-like structures are arranged into a single row, and in one species at least, *Ceratophyllus stimonsi*, there is a conspicuous patch of them.

In no instance were structures found that would suggest degenerate facets, and also there was found no definite limitation of pigment into what might be considered an ocular area. Hence, the desired proof that these structures are degenerate compound eyes appears to be wanting.

The true eyes of fleas Dahl considers as the ocelli. Their structure appears to be identical with that of ocelli, and, as far as is known, only a single cornea on one side of the head exists. Double ocelli, or ocelli with two corneas, are found in many of the mites and in many insects including degenerate forms like the biting lice. If double ocelli did exist it might be claimed by some that such ocelli were not true ocelli but only degenerate compound eyes.

Origin of Fleas from the Nematocera.

Some would derive the fleas from the dipterous suborder Nematocera. Packard and Handlirsch have advanced this view. They would derive the Siphonaptera from an ancient group from which the Mycetophilidae have developed.

If the pseudosegments of the flea antenna really represent segments, here is a strong argument for the Mycetophylid origin of fleas. There are nine of these pseudosegments in a flea's antenna, which with the addition of the first and second true segments would give an antenna of eleven segments.

The type of larva found in the Siphonaptera and the large number (eight) of abdominal segments with complete rings would favour, it appears, this view as to their origin.

A Statement of the Problem of the Origin of the Siphonaptera.

The fleas form a remarkably compact group of insects and have become highly specialized in response to their parasitic habits. Yet while highly specialized fleas differ fundamentally in the nature of this specialization from almost all other ectoparasitic groups. Instead of the whole individual being affected by the degenerative and adaptive process only certain structures have been. This fact should always be kept clearly in mind in working out any hypothesis as to their origin. Another also is of much importance. That is, that the fleas undoubtedly have all the fundamental characters of the Diptera. In the structure of the mouth-parts, in the characters of the legs, the number of tarsal segments, the number of abdominal segments, the morphology of the larvae, the loss of wings, the absence of halteres; in all these fundamental characters there is nothing that is not found in living Diptera.

Many of the generalized characters retained by the fleas are fundamental. Thus eight complete segments of the abdomen remain, with the clear evidence of nine and ten in some species. The mouth-parts are typically dipterous. The legs have the same segments as in typical flies.

It would appear to the writer that the fleas, being entirely dipterous in their characters, most probably have descended from dipterous-like ancestors. Having eight complete abdominal segments they must have descended from generalized types with as many or more complete abdominal segments. Having terrestrial larvae and being entirely parasitic on terrestrial vertebrates they should have descended from ancestors with terrestrial larvae.

The weakness of the hypothesis of Phorid descent lies above all else in the fact that an attempt is made to derive a degenerative specialized group in which the abdomen yet retains eight complete segments from forms in which only six or seven segments of the abdomen exist. This difficulty is so great and of such importance, the writer believes, that the Phorid hypothesis should be abandoned.

Probably we should look more in the direction of the blood-sucking Nematocera for ancestors of the Siphonaptera, to types with eight or more complete abdominal segments. Such types, alas, with but few exceptions, have aquatic larvae, and the structure of the adults show none of the degenerative changes so evident and characteristic of fleas. So the problem of the origin of the fleas remains; and also remains, therefore, our justification for considering them a distinct order in themselves, although their characters are admittedly those of the Diptera.

IV. Description of New Species.

Four new species are here described; one species of *Hoplopsyllus* from California, one species of *Ceratopsyllus* from Java, one species of *Phygiopsylla* from the Celebes and one of the same genus from Borneo.

Hoplopsyllus foxi, new species.

Male. A medium-sized, dark brown species.

Head about one and a half times as long as high. Region of head in front on antennal fossa very short, but high, being over twice as high as long; front vertical. Antennal fossa extending almost entirely across the head, open behind. Antenna almost completely filling fossa, club only slightly swollen and not as broad as second segment. Maxillary palpi rather short, extending beyond the tips of the maxillae by over a third their length. Maxillae with tips narrowly rounded, not acuminate. Labial palpi short, not as long as the maxillary palpi and scarcely reaching the middle of coxa I.

Thorax about as long as the head and composed of segments of about equal length. Pronotal comb with 19 spines (one broken off?), the most of which are so closely set in the row as to be contiguous and all are sharp pointed. Mesosternite large and extending far downward and forward.

Abdominal tergites without spines. Base of clasper (Pl. XIV, fig. 4) very broad and truncate above; manubrium shaped like a sled-runner with up-turned tip; upper process as broad as long and bearing about ten large setae near the tip, some of which are longer than the lower process; lower process long and slender, terminated by a stout, downwardly curved spine, bearing a very long seta on the outside near the middle, two distal to this, one on the inside and another at the base of terminal spine on the outside. Penis extending forward in the body for almost half its length beyond the tips of manubria of claspers.

Legs moderate; trochanter I reaching the tip of coxa II with both front and hind margins straight for most of their length; on the inside of coxa III near its tip is an oblique row of from 8 to 9 teeth.

Length, 2·1 mm.; height, 0·9 mm.

Type. Cat. No. 23769, U.S.N.M.

Type-locality. San Francisco, California.

Described from the holotype, a male, taken by Dr Fox from *Lepus bachmani* at San Francisco, California, in 1908.

In the United States National Museum there is a slide containing a male and female specimen of *Hoplopsyllus* which bears the determination of *Hoplopsyllus affinis* Baker by Fox. A comparison of these specimens with the types of *H. affinis* Baker (Cat. No. 6898 U.S.N.M.) shows that they are distinct. The female specimen on this Fox slide is smaller than the male, and differs in so many particulars that it is to be doubted if they are of the same species. However, this female does not agree with the female of *affinis*. The male of

the Fox specimens has the lower process of the clasper almost twice as long as the upper process, and this process bears its four setae not grouped in pairs as in the type of *affinis*. The base of the clasper in the Fox specimen is but little over half as broad as long, while in *affinis* it is almost as wide as long, also the manubria of the claspers of the two species are quite differently shaped.

Ceratophyllus javanicus, new species.

Male. A large dark brown, almost black, species.

Head about one and a half times as long as high. Frontal tubercle hyaline, triangular and partly hidden in notch. Antennal fossa extending practically across the entire head and slightly open behind. Antenna almost filling fossa; first segment swollen and broader than second; second segment with some setae as long as the entire antenna; antennal club as long as segment one and two combined and but slightly swollen. Eye small, subcircular. Ocular seta directly in front of eye and one of a row of three large setae on the front region of head. Above the antennal fossa is a patch of minute setae. Segments I and II of maxillary palpi subequal. Maxillae ending in long spine-like points. Beak twice as long as maxillae and reaching base of coxa I.

Thorax long, slender. Prothorax slightly over one-half as long as high; pronotal comb of twenty spines. On each side prothorax has a vertical row of six long setae which alternate with minute setae.

Abdomen strongly up-curved, thickest at the fourth segment. Caudal flaps very large, slightly longer than broad. Clasper with a long slender process having subparallel margins; finger almost enormous, expanding, rectangular, slightly longer than broad and with a single stout spine at lower angle. Tip of ninth sternite (Pl. XIV, fig. 5) ending in a long spatula-like process with an external seta-bearing carina and an internal up-turned hooked process. Eighth sternite (Pl. XIV, fig. 5) with a dorsal comb of about a dozen, long, curved setae, the most distal of which is the stoutest and twice curved. Below, the eighth sternite has an enormous seta situated near the proximal end of comb of seta and slightly anterior to this a trifid seta of moderate length.

Legs long; trochanter I falling slightly short of tip of coxa I; trochanter II reaching to about the middle of coxa III.

Length, 2·9 mm.; thickness of abdomen, 1·2 mm.

Female. Much larger than male, the abdomen particularly being larger and not up-curved. Receptaculum seminis (Pl. XIV, fig. 5) worm-like, the head being scarcely larger than the tail and shorter. Stylet about three and a half times as long as broad. Its seta twice as long as the stylet itself.

Length, 4·0 mm.; greatest thickness, 1·6 mm.

Type. Cat. No. 23770, U.S.N.M.

Type-locality. Fjibodas Mt., Gede, Java.

Description based upon 1 male and 11 females collected by Palmer and Bryant, September 12, 1909 from skunk, Fjibodas Mt., Gede, Java.

Although differing from any described species in the characters of the male genitalia this species clearly belongs to the oriental *fimbriatus* group which has the eighth sternite of the male greatly modified.

Pygiopsylla celebensis, new species.

Male. A medium-sized species; colour light chestnut, or yellowish brown; ctenidium and eyes black.

Head but slightly longer than high, front recurved. Antennal fossae long, extending almost the whole length of the head and to the posterior margin where they are barely closed. Antennae themselves almost filling the fossae; first segment geniculate; second a cupped disc with about a half dozen small, parallel marginal setae; pseudosegments nine in number. Eye not projecting into antennal fossa, crescentic. Genal setae of posterior row, four; second from the top shortest. Beak reaching to the distal end of trochanter I; last segment of labial palpi slightly longer than next to last, which is subequal to antepenultimate.

Thorax slightly longer than the head. Prothorax shorter than the mesothorax; ctenidium with fourteen teeth, all of which are subequal, except for the ventral ones which are slightly smaller. Mesothorax broader than either the pro-, or metathorax and overlapped for about one-half its length by the pronotal ctenidium. Metathorax with anterior margins concave and posterior convex.

Abdomen very much narrowed posteriorly, broadest at the third segment. Ninth sternite L-shaped (Pl. XIV, fig. 6), distal one-third provided ventrally with about a dozen commixed short setae and spines and almost twice as many stubby spinules. Penis extending to the posterior margin of segment V, large and canoe-shaped. Finger over one-half as broad as long, upper margin strongly convex except at the base where it is emarginate, lower angle of finger broadly rounded and bearing four or five moderate setae. Clasper squarish with long manubrium.

Legs moderate. Tibia III with three setae in outer posterior row and four setae in outer anterior row and a single seta in front of outer anterior row. First segment of tarsus III equal to second and third taken together; fourth but slightly longer than broad; the last equal to the third.

Length, 1·8 mm.; height of abdomen, 0·6 mm.

Type. Cat. No. 23771, U.S.N.M.

Type-locality. Pulo Sembeh, Celebes.

Description based on the holotype, a male specimen taken from the skin of *Sciurus evidens* (U.S.N.M. 217811).

Female not known.

This species is most nearly related to *P. solida* Rothschild. It differs from Rothschild's species in having a beak that extends to the tip of trochanter I instead of falling short of this trochanter; in having the distal third of the ninth sternite of male armed with many very short, stubby spines in addition

to short spines and setae; in the shape of the finger, which is much broader; in having four or five short setae at the lower angle of finger instead of three long ones; and in some other characters.

Pygiopsylla sciuri, new species.

Female. A medium-sized, yellowish brown species, with eyes and ctenidium black.

Head almost as high as long, front slightly recurved. Antennal fossae extending from the postero-ventral margin of the head three-fourths the way across the same. Antennae almost filling fossae; club of each about twice as long as broad; second antennal segment with about six dorsal, marginal setae that do not reach the middle of the club. A vertical row of long setae extends along near the anterior margin of the antennal fossa. On the border of the fossa both above and below are situated two indistinct rows of very short setae. Three long setae on the posterior margin of the head. Eyes stoutly crescentic. Maxillary palpi but little over one-half again as long as beak, last segment of each, half again as long as penultimate. Labial palpi reaching almost to the tip of first trochanters.

Thorax as a whole much longer than the head, the pro- and mesothorax together being about as long as the latter. Teeth of pronotal comb longer than the pronotum itself and overlapping mesonotum by two-thirds their length, those in the middle equal to the ventral ones except for the last ventral tooth which is smaller. Mesothorax about twice as long as prothorax. Metathorax intermediate in length between the pro- and mesothorax.

Abdomen about one and a half times as long as broad and broadest at the fourth segment. Posterior border of seventh sternite only very slightly emarginate (Pl. XIV, fig. 7); incrassation in front of emargination. Tenth sternite with a prominent, ventral protuberance near the base which is squarish and tipped with about half a dozen setae of various lengths. Stylet surpassing tip of tenth sternite by about one-half the length of its distal segment. Head of receptaculum seminis irregularly squarish, as long as the curved, sausage-shaped tail.

Legs moderate; tip of trochanter I not quite reaching the base of trochanter II; tip of trochanter II reaching to about the middle of femur III. Anterior row of lateral setae of tibia III consisting of four setae, posterior row of four or five.

Length, 2·3 mm.; thickness of abdomen, 0·8 mm.

Type. Cat. No. 23772, U.S.N.M.

Type-locality. Borneo.

Description based on the holotype, a female specimen, taken from the skin of *Sciurus atricapillus* (U.S.N.M. 198774).

This species comes next to *P. hoplia* Jord. and Roths., but differs from *hoplia* in having a much more shallow emargination on the posterior border

of the seventh sternite of female, in having a differently shaped receptaculum seminis and a differently shaped tenth sternite of the female.

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EXPLANATION OF PLATE XIV.

Fig. 1. Left clasper of male of *C. niger*, above to left; same of *C. gallinae*, below at left; receptaculum seminis of *C. niger*, above at right; same of *C. gibsoni*, below at right. Drawings not to same scale; that of receptaculum seminis of *C. gibsoni* is from type specimen.

Fig. 2. Side view of genal region of the head of type species of *Catallagia* Rothschild, *C. charlottensis* Baker.

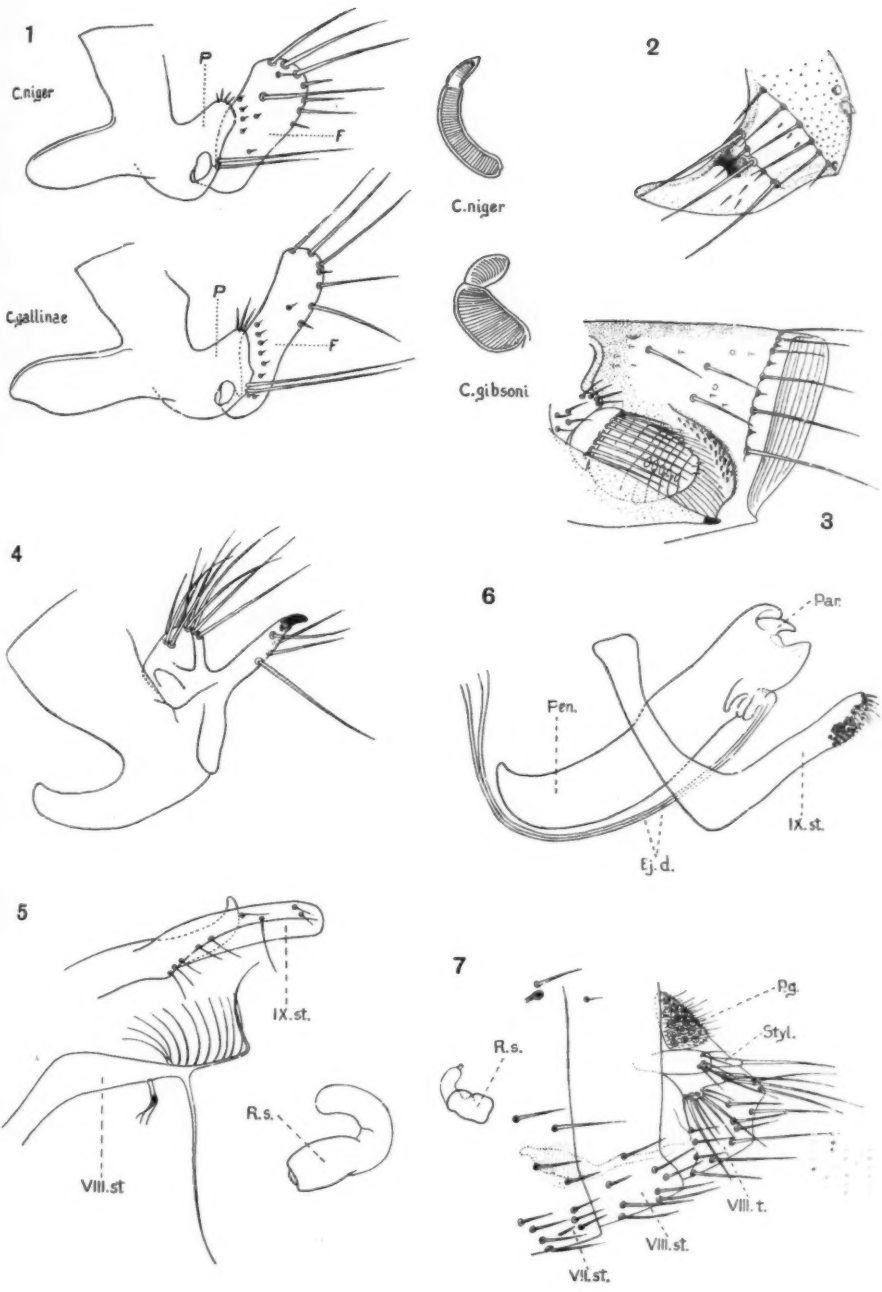
Fig. 3. Posterior region of the head of the male of *Ctenocephalus canis* Curtis.

Fig. 4. Clasper of male of *Hoplopsyllus foxi*, new species.

Fig. 5. Eighth and ninth abdominal sternite of male and receptaculum seminis of female of *Ceratophyllus javanicus*, new species.

Fig. 6. *Pygiopsylla celebensis*, new species. Ninth sternite and penis of male. *Ej.d.*, ejaculatory ducts; *par.*, paramere; *pen.*, penis; *IX. st.*, ninth sternite.

Fig. 7. *Pygiopsylla sciuri*, new species. Tip of abdomen of female. *P.g.*, pygidium; *R.s.*, receptaculum seminis; *Styl.*, stylet; *VII. st.* and *VIII. st.*, seventh and eighth sternites, respectively; *VIII. t.*, eighth tergite.


1
C.niger
P
F
C.gallinae
P
F
C.niger
C.gibsoni
2
3
4
5
IX.st.
R.s.
VIII.st
6
Par.
Pen.
IX.st.
Ej.d.
7
Pg.
Styl.
R.s.
VIII.t.
VIII.st.
VII.st.